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P-Hydroxyphenacyl Group: Potential and Efficient Biocompatible Photoremovable Protecting Group for Controlled delivery of Drug.

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ABSTRACT

α-keto caged p-hydroxyphenacyl group (pHP) an excellent photoremovable protecting group due to its fast and efficient release of substrate molecules and formation of photochemically cleaned water soluble byproduct following photo-favorskii rearrangement. Researchers are very carefully analyses the chemical properties and modified in such a way that the absorption is enhanced from 300 nm to 700 nm. In this review article we have minutely summarized the photophysical and photochemical properties and in vitro studies of all pHP-containing group.

Keywords: Photoremovable Protecting Groups, Photoirradiation, Parahydroxyphenacyl, Control delivery, Photoproduct.

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INTRODUCTION

Photoremovable protecting groups (PRPGs) have attracted great deal of attention because of their light stimulated controlled release ability of cage substrate on exposer to light [1]. Scientists have utilized the PRPGs in a wide application field like functional group protection in organic synthesis, photolithography, sensing, drug delivery and synthesis of light responsive organic materials [2-3]. There is a list of criteria those should be follow during designing of a new ideal PRPG and those are [4]

i) The designed PRPG should absorb the light wavelength of well above 300 nm to avoid damage in biological system
ii) The designed PRPG should have clean photoreaction and should have high quantum efficiency
iii) The formed photochemical by-product in photoreaction should be transparent and bio-compatible in nature.
iv) The designed PRPG should have the ability to dissolve in physiological medium and capacity to pass through the biological membranes (e.g. cell membrane, blood brain barrier etc.) [5].

Among the known photoremovable protecting groups, aqueous soluble second generation α-keto caged p-hydroxyphenacyl group (pHP) proves its excellence in all respect and most of the scientists accepted that phenomena [6]. It proves its excellence in vary fast (with in a few nanoseconds after excitation) and efficient release of the substrate molecules. In the early days of discovery of pHP group, it has been used in neurobiology and enzyme catalysis [7]. The most fascinating nature of pHP group is that the photorelease of substrate happens via photo-favorskii rearrangement and formed water soluble photochemically cleaned p-Hydroxyphenacyl acetic acid as a photoproduct. Given's group showed the release of GABA and Glutamate from the substrate like methoxy, trifluoromethyl or trifluoro methoxy on the para-position of the pHP group [7]. They have observed the improvement in photorelease process than the parent pHP group. Given's et. al also demonstrated the release of Adenosine triphosphate (ATP) from pHP group [6]. They observed that the quantum efficiency of the above-mentioned process was 0.37 ± 0.01 and the rate constant was $(5.5 \pm 1.0)10^{8}$ s^{-1}. Givens et al also studied and demonstrated the photorelease of oligopeptides and bradykinin from pHP group [7].

Observing this entire phenomenon Andeson and Reese state out the essential four steps of the photorelease process [8]-

1. 1st Step: At first, upon excitation, pHP ester goes to the singlet state followed by triplet state via rapid ISC (inter system crossing process).
2. 2nd Step: Abstraction of proton from phenolic-OH Group in triplet excited state.
3. 3rd Step: Formation of spirodienedione intermediate following photo-favorskii mechanism followed by release of substrate.
4. 4th Step: Finally, formation of rearranged photoproduct p-Hydroxyacetic acid by the hydrolytic ring opening of spirodiketone.

pHP groups showed its excellence in the uncagging of biomolecules due to their salient features like-

i) Very clean and fast substrate release process
ii) Excellent photochemical quantum yield or efficiency
iii) Synthesis process is easy
iv) Its small size gives the opportunity to install easily in any substrate
v) Biocompartible and transparent photoproduct

Instead of such a captivative properties of pHP group, it remains unexplored in the theranostics applications because of two boundaries- first pHP group can't absorb light in visible region (≥ 410 nm) and secondly, non-fluorescent nature. Many research groups have tried and successfully modify the pHP group by elevating absorption the group to the visible wavelength region and introducing fluorescence property into it and also kept the salient features of pHP group intact (**Scheme 1**).

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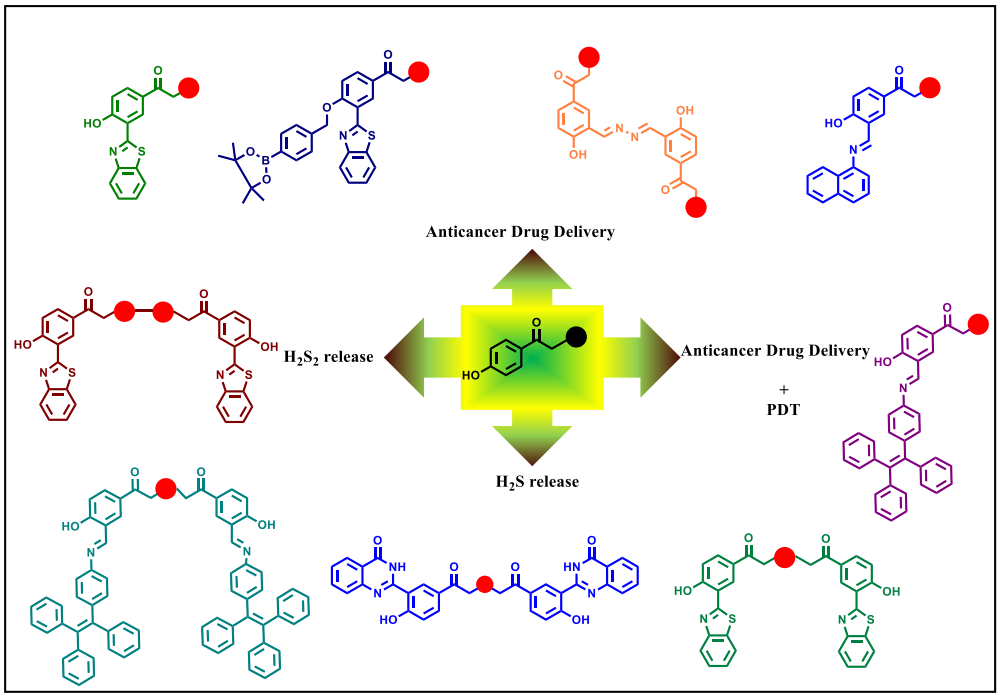
Scheme 1: Upgradation of pHP group and its application in various field.



Anticancer Drug Delivery

A p-Hydroxyphenacyl–Benzothiazole–Chlorambucil Conjugate as a Real-Time-Monitoring Drug-Delivery System Assisted by ExcitedState Intramolecular Proton Transfer

One of the beautiful explorations of parahydroxy group was carried by barman et al[9] simply by appending benzothiazole group into it and this gives the amazing advantages like-

1. Extension of absorption into visible region
2. In-buit ESIPT process makes the molecule environment sensitive and fluorescent
3. ESIPT assisted deprotonation of the phenol moiety and helps for faster release of drug molecule
4. Distinct fluorescence colour change during drug release.

Due to the presence of ESIPT effect pHP-Benz-Cbl two emission bands with two emission maxima, one at 515 nm (keto form) and second at 450 nm (enol form) was noticed in polar protic (ETOH, MeOH) and polar aprotic (Acetonitrile, Tetrahydrofuran) solvents (Figure 1b). This compound showed only one band at 510 nm in non-hydrogen bonding solvents (cylohexane, benzene) and this band is corresponds to the keto form of the molecule. Photorelease of the compound (Figure 1c) was analyzed in acetonitrile/HEPES buffer (1:19) taking 20 mL 1 x 10^{-4} M solution. The solution was exposed to visible light (≥ 410 nm) using 125 W medium pressure mercury lamp (UV cut off filter 1 M $NaNO_2$ solution). Reverse phase HPLC overlay chromatogram showed the drug release efficiency of pHP-Benz-Cbl upon photo irradiation and 15 min time was required to release 90% of drug from the substrate molecule pHP-Benz-Cbl. The molecule showed a quite high quantum efficiency (0.46). Time dependent DFT calculation revealed that ESIPT process is happening from ground state singlet state. This study also supports the photo release mechanism, that is, proton transfer from singlet state (ESIPT Process) followed by ISC and triplet state photo-favorskii rearrangement (Figure 1f). Most –interesting factor of this molecule is distinctive fluorescence color change from green to blue during photo-release (Figure ei). Emission spectral graph at different irradiation time showed the gradual diminishes of green emission at 517 nm and with concombinet increment of blue shifted blue emission band at 450 nm (Figure eii). Cellular internalization also proved the real-time monitoring ability of pHP-Benz-Cbl molecule (Figure 1d).

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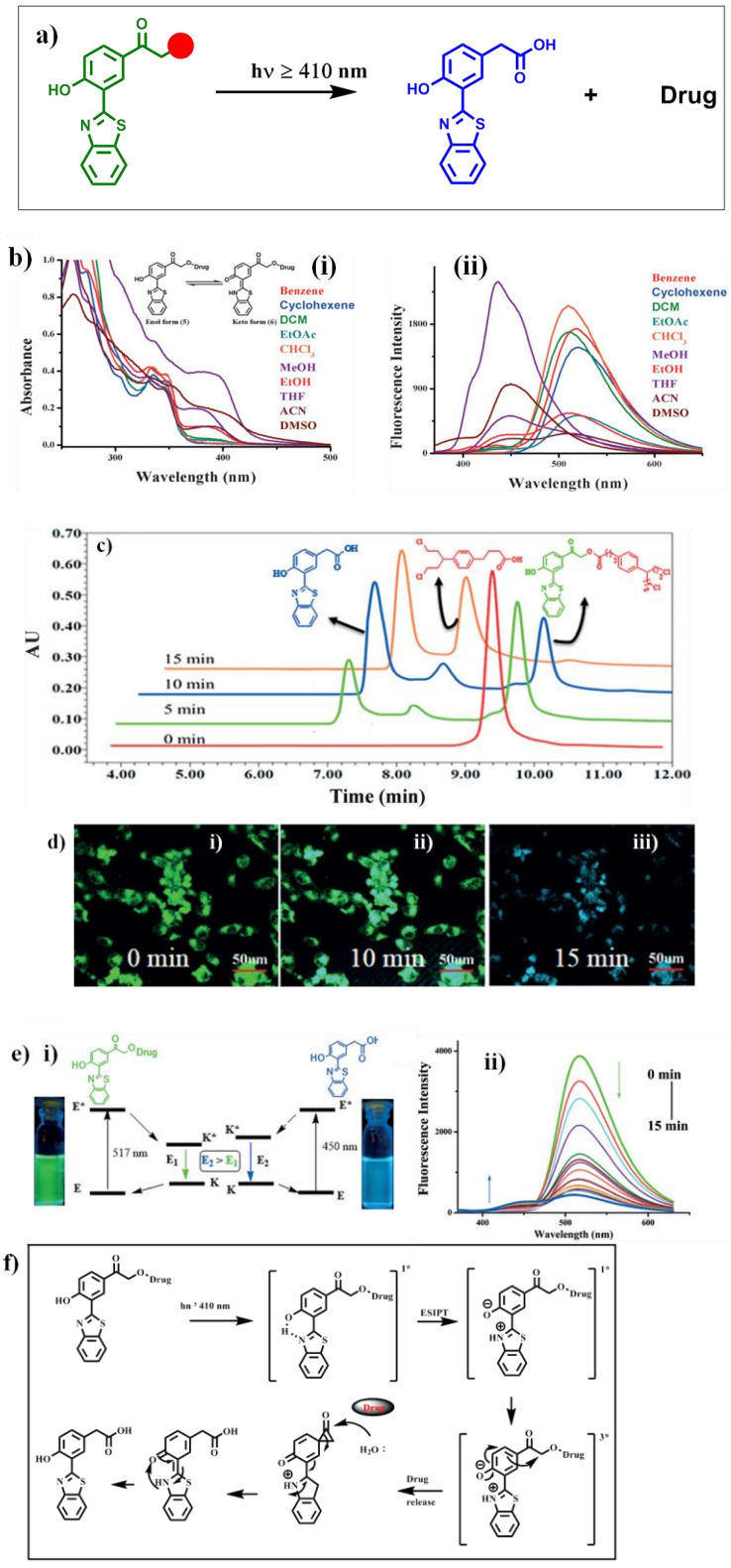

Figure 1: a) Outline of drug release process from pHP-Benz-Cbl molecule upon visible light (λ≥ 410 nm) irradiation; b) (i) absorption and (ii) emission spectral graph of pHP-Benz-Cbl molecule in different solvent system; c) Reverse Phase HPLC chromatogram for the photolysis process of pHP-Benz-Cbl molecule; d) Confocal image of HeLa cell line treated with pHP-Benz-Cbl molecule at (i) 0 min; (ii) 10 min and (iii) 15 min visible light irradiation; e) Investigation of real time monitoring process (i) by energy Diagram and, (ii) by spectrophotometry; f) ESIPT assisted drug release mechanism process of pHP-Benz-Cbl molecule. (b-fd) is reprinted with permission from ref. Copyright 2018: wiley.

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Environment Activatable Nanoprodrug: Two-Step Surveillance in the Anticancer Drug Release

Biswas et al [10] had successfully synthesized boronate coupled benzothiazole-pHP-Chlorambucil conjugate, which has two steps-controlled step 1- control in detection of reactive oxygen species [11-16] which is overproduced in cancer cell, and step-2 – control in drug release process (Figure 2a) [17-23]. They have synthesized nanoparticle from boronate coupled drug conjugate and named H_2O_2 –activatable nano prodrug, ANPD-X. Synthesized nanoparticles were globular in shape with average size of ~78 nm. Nanoparticles had absorption above 410 nm and emission maxima at 448 nm. H_2O_2 assisted activation of ANPD-X was examined by addition of 50 equivalent of H_2O_2 and proved by the dramatic increment of emission band at 518 nm due to enabling the ESIPT process (keto emission) (Figure 2b).

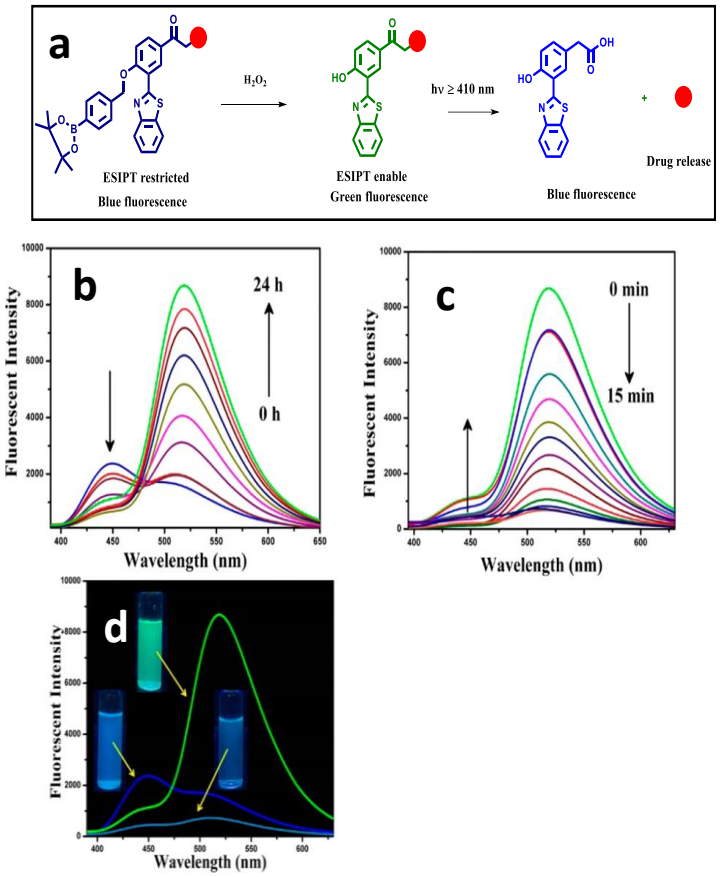

Figure 2: a) Schematic representation of two steps Surveillance and drug release process; b) Enabling of ESIPT process upon incubation of ANPD-X with H_2O_2 in different time interval; c) Fluorescence spectral graph at different irradiation time; d) Fluorescence response with presence of H_2O_2, absence of H_2O_2 and after 15 min of irradiation. (b-d) is reprinted with permission from ref. Copyright 2017: American Chemical Society.

For the examination of second control, which is photo responsive drug delivery ANPD-X nanoparticle at 1x 10^{-4} (M) was treated with 300 equivalents of H_2O_2 and kept 24h and then exposed the solution to visible light (≥ 410 nm) for 15 min (Figure 2c). RP-HPLC study showed that 15 min is required for complete drug release. The interesting factor about the boronate coupled drug conjugate is that the system showed two times fluorescent colour changes-(i) blue to green during H_2O_2 activation due enabling of ESIPT process and (ii) Green to blue during photo release due to production of photoproduct via photo favorskii mechanism (Figure 2d). To check the fluorescent switching ability ANPD-X was incubated with breast cancer cell line HeLa for 12 h and switching of blue to green fluorescence was observed in confocal image study. Cellular study also showed the second fluorescence colour change from green to blue after photorelease.

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p-Hydroxyphenacyl conjugated salicylaldazine: single component photoresponsive fluorescent organic nanoparticles showing 'AIE + ESIPT' assisted photorelease for dual anticancer drug delivery with real-time monitoring ability.

Fluorogens having ability of aggregation-induced emission (AIE)[24-25], have capture a great deal of attention in recent time due to its high signal to noise ratio, good photostability in the solution phase and its most interesting features which is brighten in the aggregated state[26]. AIE molecules showed very weak florescence in solution phase and it's fluoresce enhanced many fold in aggregated state. Their photophysical behavior completely depends on restricted intramolecular motion in the aggregated state. AS per as we know Biswas et al first developed AIE based organic nanoparticles as a DDS using pHP (p-hydroxyphenacyl) group as a phototrigger. The excellence of the photoresponsive nano DDS is that can exhibit dual phenomenon AIE and ESIPT by simply anchoring salicylaldazine moiety into a pHP phototrigger[27] (Figure 3a). Newly designed nano DDS (nanoparticulate drug delivery system-5, NDD5) showed attractive features like

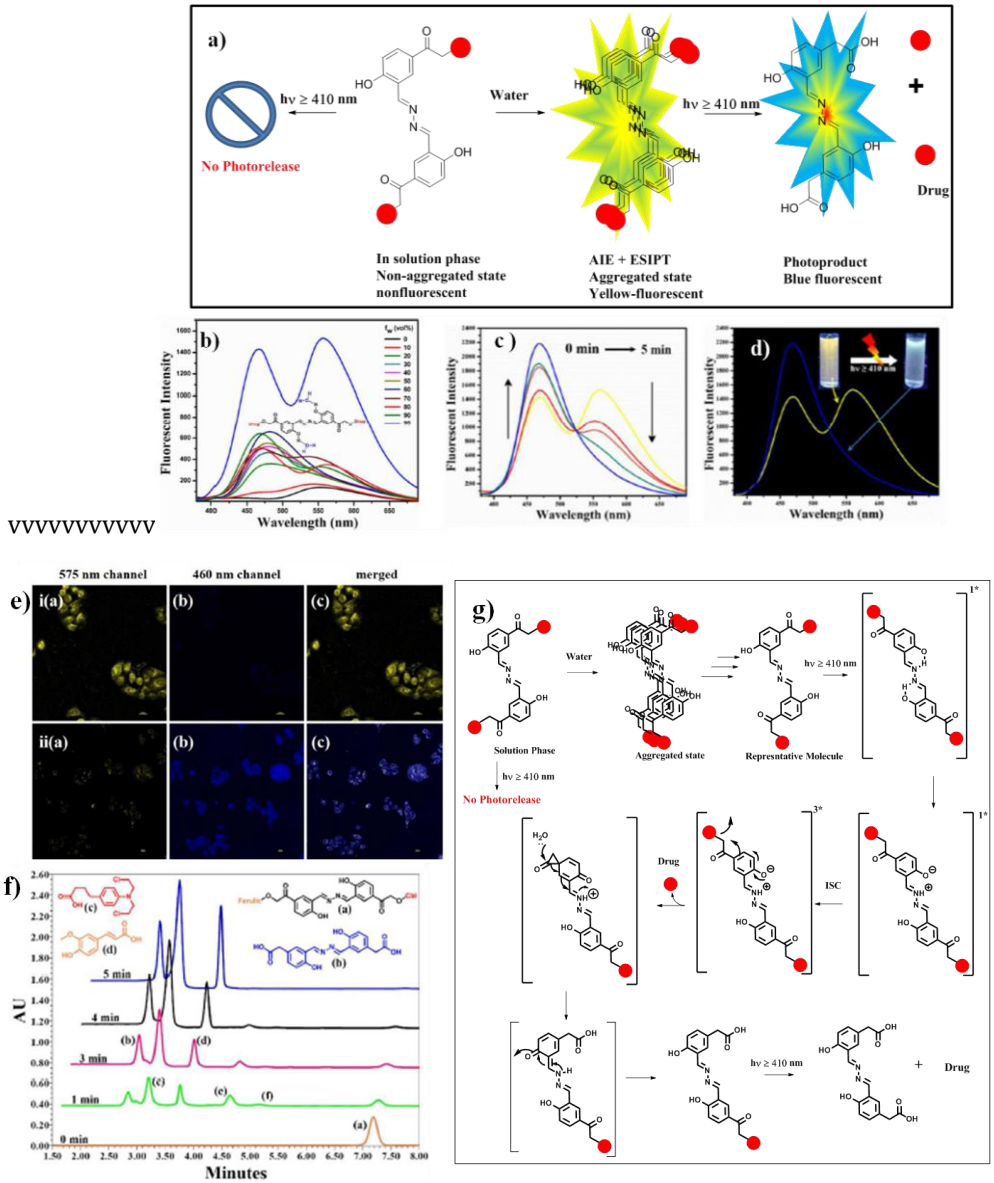

Figure 3: a) Schematic representation of 'AIE and ESIPT' assisted dual drugs release from p-Hydroxyphenacyl conjugated salicylaldazine ; b) Evidence of AIE process; c)Fluorescence graph of photolysis process; d) distinct fluorescence colour on drug release; e) Confocal image of NDDS-5 (i) before and (ii) after photoirradiation; f) HPLC chromatogram profile (a-NDDS-5, b-photoproduct, c-chlorambucil and,d-ferulic acid; g) Dual drugs release mechanism. (b-g) is reprinted with permission from ref. Copyright 2018: Royal Society of Chemistry.

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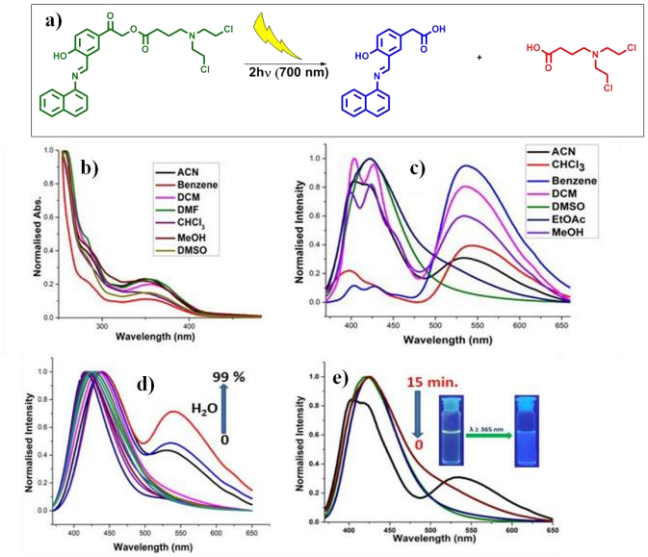
(i) Extended excitation wavelength in visible region (greater than 410 nm)
(ii) Drug release ability only in the aggregated state
(iii) Produces a distinct fluorescence colour on drug release, thus providing a scope to track the drug release profile in realtime (Figure 3d) and
(iv) Sequentially dual (different) drug release ability so that it can be used for combination chemotherapy (Figure 3f)

The organic nano DDS was globular in shape, analysed by TEM (Transmission electron microscopy) and the size of the nanoparticle (NDDS5) was around 68 nm and these nanoparticles were quite stable in the dark in culture medium but almost 90% of the caged dual drugs (chlorambucil and ferulic acid) were released upon photoirradiation of visible light for 5 min of duration (Figure 3c). In cell culture medium (HeLa cell line) nano NDDS5 showed cytotoxicity only upon visible light irradiation and the result clearly showed by MTT assay.

To sum things up, nano DDS had excellent biocompartibility, great cellular uptake capability and most interstingly spatiotemporal precision of the drug release, these makes nano NDDS5 a fabulous photoresponsive nano DDS for dual drug delivery (Figure 3g).

Two-photon responsive Napthyl tagged p-hydroxyphenacyl based drug delivery system: uncaging of anti-cancer drug in the phototherapeutic window with real-time monitoring

Photo-uncagging of chlorambucil from p-Hydroxyphenacyl phototrigger upon photo exposer in the region of 650-950 nm is carried out by A. Singh et. al [28]and the process has been done by tagging two photon absorbing Napthyl group with p-Hydroxyphenacyl based drug delivery system. Their synthesized molecule has the ability to show photon absorption in the therapeutic window (700 nm),[29-33] exhibits AIE Phenomenon, large shifted emission (presence of ESIPT process) and, real time monitoring upon photo-release of drug molecule (Figure 4a). pHP-Naph-Cbl is very much solvent sensitive and it showed dual emission in polar-protic solvents λmax at 425 nm and 550 nm (Figure 4c) and responded Aggregation Induced Emission (AIE) phenomenon when emission spectra (conc. 1 x 10-4 M) of pHP-Naph-Cbl was recorded in ACN-H_2O mixture (Figure 4d). They observed abrupt increment in emission intensity when the water fraction increased from 85-95% (V/V). pHP-Naph-Cbl molecule is quite stable in biological pH 7.4. pHP-Naph-Cbl showed promising photorelease ability upon exposer to one photon (λ ≥ 365 nm) and two photon (λ = 700 nm). One photon light irradiation took 15 min for 90% of drug release (Figure 4e-f) while pHP-Naph-Cbl took 3h of two photon light irradiation for 25% of drug release. For drug release pHP-Naph-Cbl molecule follow the photo-Favorskii rearrangement , spirodiketone intermediate and rearranged photoproduct formation , exactly like its parent molecule pHP. During photo release process a distinct fluorescence colour change was observed from greenish yellow to blue in spectrophotometer and confocal image (Figure 4h). Hence, the two photon absorbing DDS has TPA cross-section ≥ 20 GM and excellently uncage drug with TP uncagging of 10 GM at 700 nm (Figure 4g).



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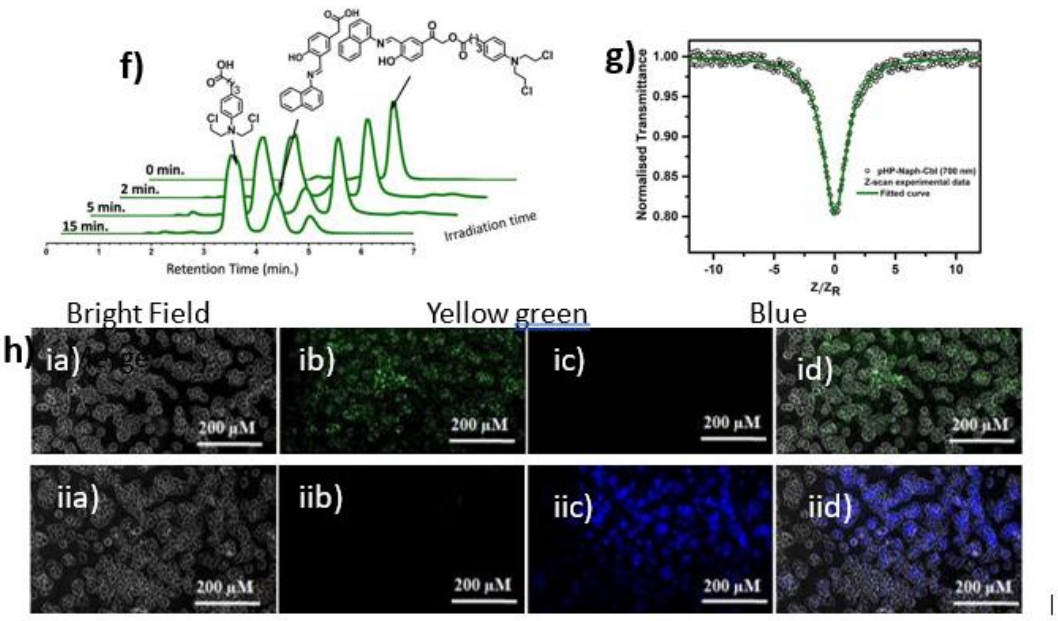
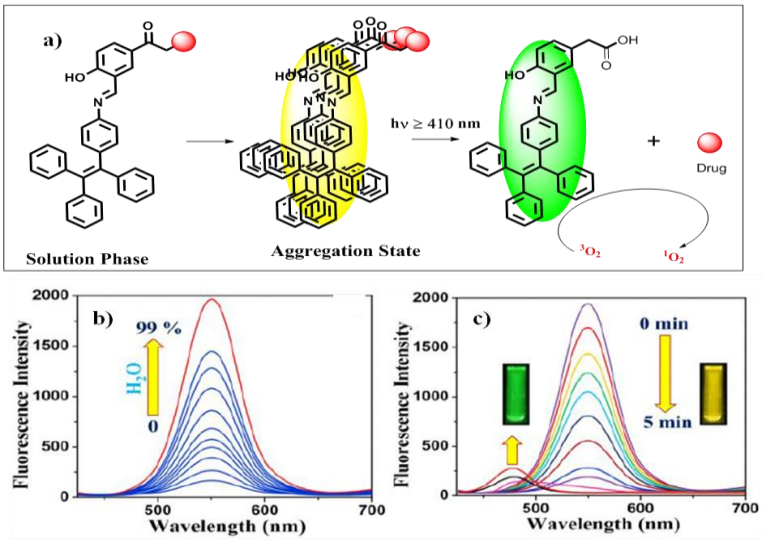


Figure 4: a) Schematic representation of drug release from pHP-Naph-Cbl in therapeutic window; b) Absorption and c) emission spectra of pHP-Naph-Cbl in different solvent system; d) Record of fluorescence spectral behavior change with increased amount of water fraction; e) Different fluorescence spectral peaks observed in spectrophotometer during photolysis; f) HPLC chromatogram showed 15 min is required for complete photolysis; g) Z- scan measurement for pHP-Naph-Cbl at λ=700 nm; h) cellular internalization study (confocal images) a) bright field, b) yellow green field, c) blue field, d) merge image and, i) 0 min of light irradiation, ii) 15 min of light irradiation. (b-h) is reprinted with permission from ref. Copyright 2022: American Chemical Society.

Anticancer Drug Delivery + PDT

Visible Light Triggered Fluorescent Organic Nanoparticles for Chemo-Photodynamic Therapy with Real Time Cellular Imaging

The advantages of AIE + ESIPT in one system had been nicely explained by Biswas et al[27] and in continuation, partiban et al[34] had developed an AIE based nanosystem named TPE–pHP–Cbl NPs by stitching tetraphenylethylene moiety with p-hydroxyphenacyl (pHP) phototrigger for the combination therapy (Photodynamic Therapy (PDT) [35-36] and Drug delivery (Figure 5a).



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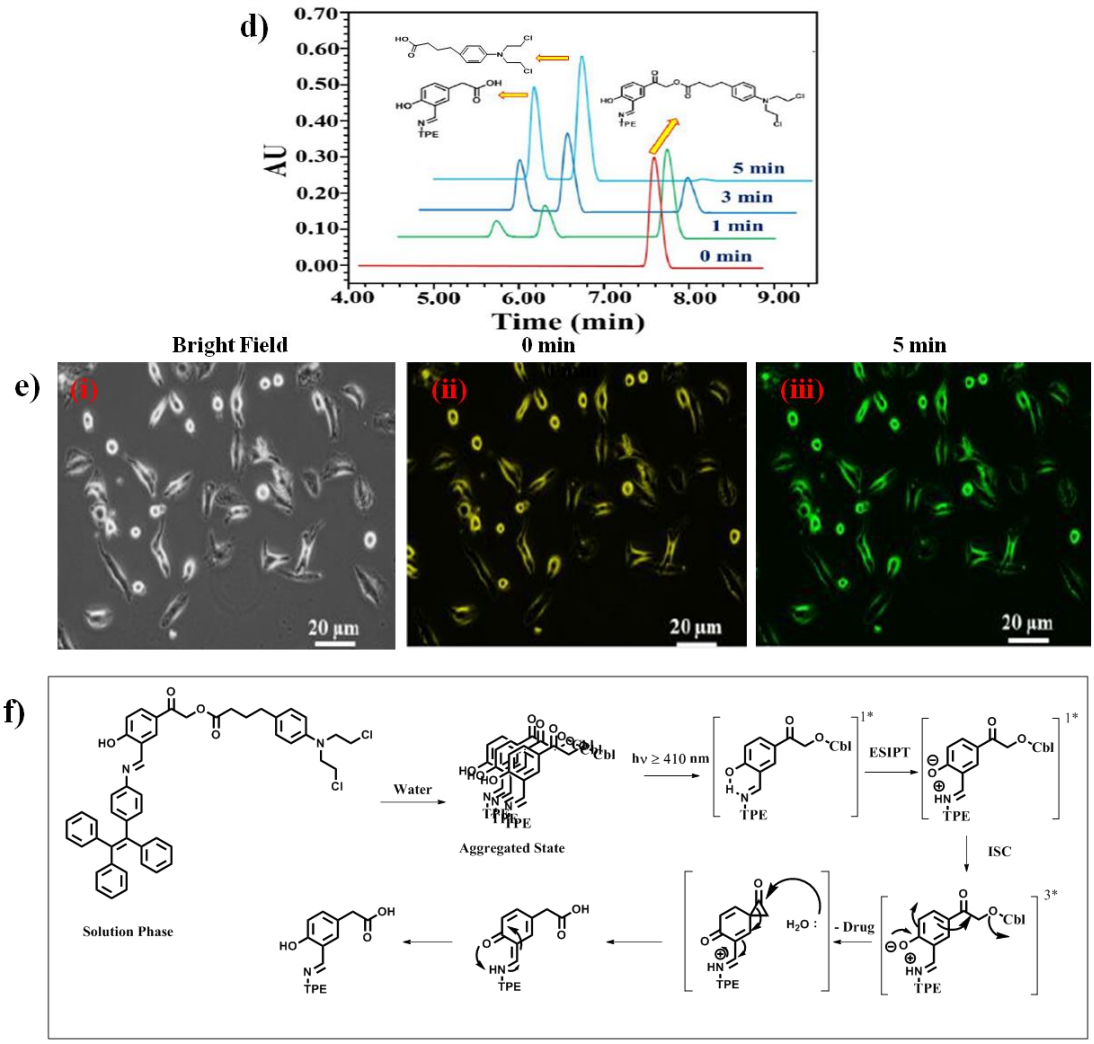

Figure 5: a) Schematic representation of combination therapy of TPE–pHP–Cbl NPs; b) Enhancement of fluorescence intensity upon aggregation;c) Gradual disappearance of yellow fluorescence and appearance and increment of green fluorescence with increase of irradiation time; d) Overlay chromatogram for the photorelease of TPE–pHP–Cbl naoparticle; e) Confocal images of TPE–pHP–Cbl naoparticle incubate cell (i) in bright field; (ii) 0 min of light irradiation and; (iii) 5 min of light irradiation. (b-f) is reprinted with permission from ref. Copyright 2018: American Chemical Society.

The re-precipitation technique was used to prepare TPE–pHP–Cbl naoparticle and synthesized anoparticles were spherical in shape and the average size was around 38 nm. Due to the presence of AIE phenomenon yellow fluorescence was observed and gradually increased with increasing water fraction in the solution (Figure 5b). The spectral study revealed that the highest brightness of fluoresce was observed when the water fraction was 99%. The real time monitoring capacity was demonstrated by intimating fluorescence colour change from yellow to green on photolysis. The result was supported by blue shifted spectral graph (Figure 5c). In a similar fashion, drug release profile was analysed by Revese phase HPLC study and the chromatogram visualized that only 5 min visible light irradiation is required to release almost 90 % of drug (Figure 5d). The singlet oxygen generation quantum yield was quantified and those were 0.28 and 0.24 for TPE–pHP–Cbl NPs and TPE–pHP–OH NPs (photoproduct), respectively. In confocal image (Figure 5e), the fluoresce colour change was beautifully observed, Yellow before irradiation and green after irradiation. The appearance of green fluoresce on photolysis is due to the disruption of conjugation (Figure 5f). The most interesting features of the TPE–pHP–Cbl NPs are the real-time monitoring ability during drug release, and single oxygen generation, which further enhance the PDT activity.

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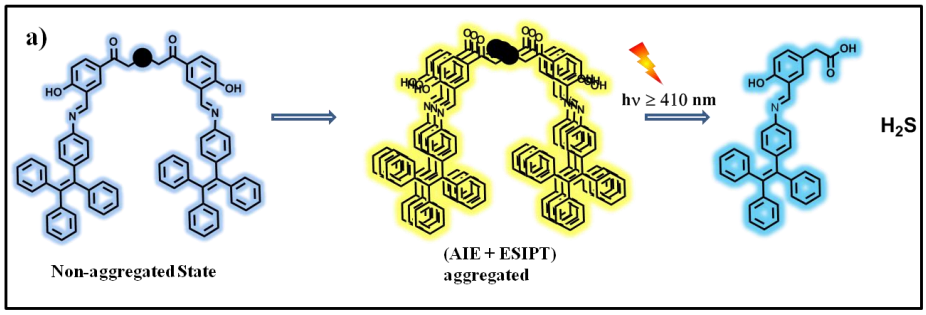
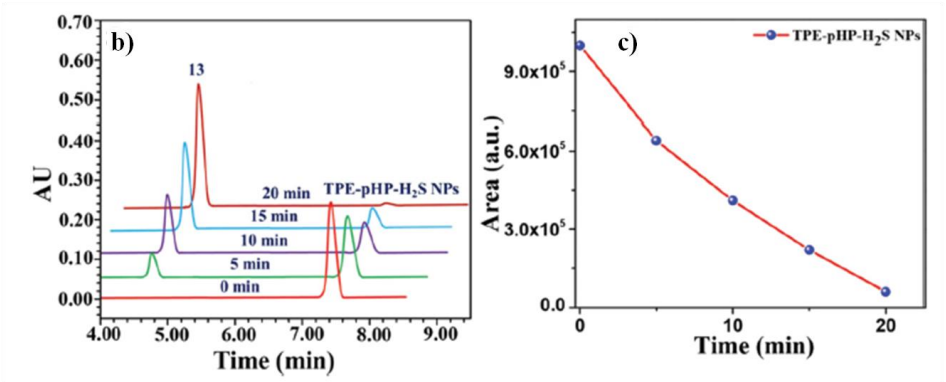
Hydrogen Sulfide (H_2S) Release

Tetraphenylethylene conjugated p-hydroxyphenacyl: single component fluorescent organic nanoparticles for the release of hydrogen sulfide with real-time cellular imaging

Scientific community had perceived its blessing hands towards H_2S as an effective gasotransmitter[37-41] in last few decades. H_2S spread its blessing hands in physiological problems like oxidative stress, inflammation, vascular tone and angiogenesis. H_2S has concentration dependent activity: (i) Cell protective nature at lower concentration, and (ii) exhibit cell damaging ability in higher concentration. Hence, for treatment purpose it is necessary to release H_2S in a regulated way. In this time also parthiban et al offer an excellent visible light activatable single FONPs by sewing up tetraphenylethylene (TPE) group with p-hydroxyphenacyl (pHP) photoresposive group for the regulated release of H_2S with real time monitoring ability (Figure 6a). The beneficial characteristics offered by synthesized TPE–pHP–H_2S NPs are (i) enhanced aggregation-induced emission, (ii) no reabsorption due to large stokes shift, (iii) absorption wavelength is in visible region (> 410 nm), (iv) photorelease of H_2S only in aggregated state (Figure 6di), (v) determine the complete release of H_2S from the fluorescence colour change (real time monitoring) and lastly not the least (vi) no use of external reagent for the release of H_2S. TPE–pHP–H_2S conjugate was dissolved in acetonitrile and TPE–pHP–H2S NPs were prepared by applying reprecipitation technique. Synthesized nanoparticles were quite good in size (~ 35 nm) and shape was spherical (showed in HR-TEM). Photolysis of TPE–pHP–H_2S NPs was carried out under visible light treatment in aggregated sate (Figure 6b). Parent pHP group follow the ESIPT assisted photo-Favorskii mechanism for H_2S release. Methylene blue assay was carried out to check the H_2S release ability and the result showed that 100 mM of TPE–pHP–H_2S NPs has ability to release ~40 mM of H_2S upon 20 min of irradiation (Figure 6c). Complete H_2S release was sorted by observing fluorescence colour change from yellow to blue (Figure 6dii).





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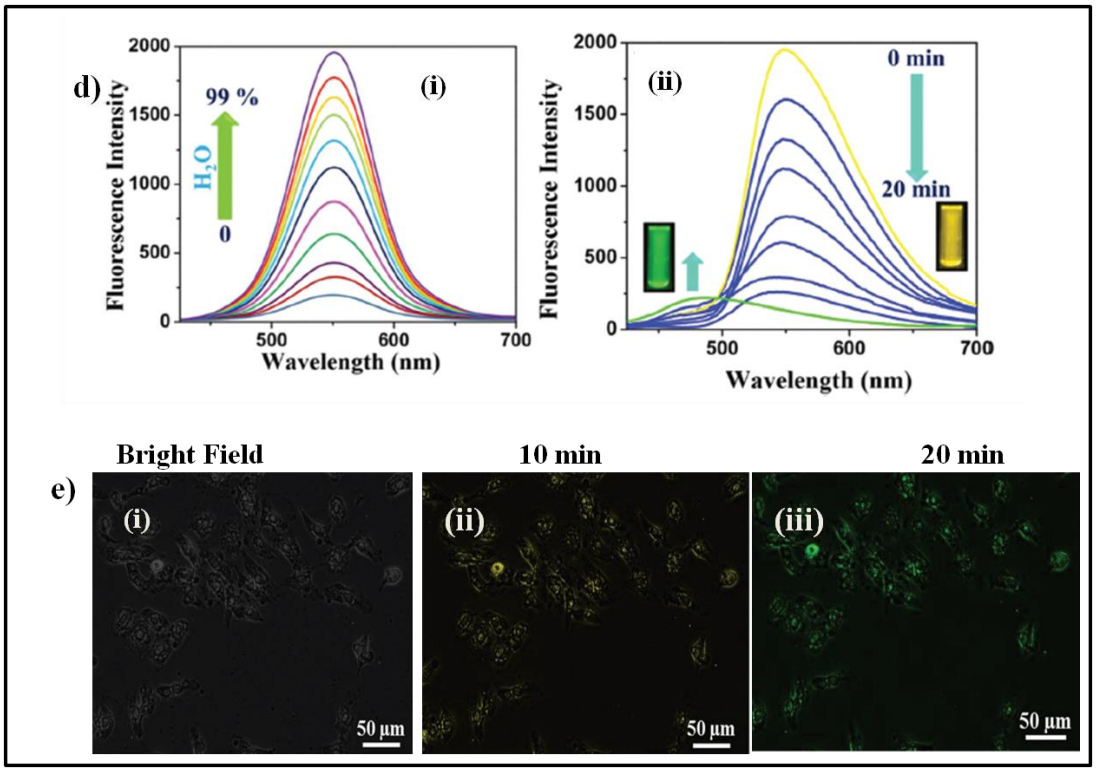

Figure6: a) 'AIE + ESIPT' assisted H_2S release from TPE–pHP–H2S NPs (Schematic); b) Photorelease study from reverse phase HPLC ; c) Amount of H2S release with respect to time study from the HPLC area; d) (i)Enhancement of aggregation and fluorescence intensity with the increment of water fraction; (ii) fluorescence spectral change during photolysis; (e) Confocal image study (i) Bright Field, (ii) 10 min of photo irradiation and (iii) 20 min of photo irradiation of TPE–pHP–H2S NPs. (b-e) is reprinted with permission from ref. Copyright 2018:Royal Society of Chemistry.

In vitro study (Figure 6e) using cervical cancer cells proved the good cell uptaking capability and real time monitoring ability. MTT assay demonstrated that TPE–pHP–H_2S NPs are non-cytotoxic at the cell culture concentration and TPE–pHP–H_2S NPs can be used for further cell study. Hence, TPE–pHP–H2S NPs is an excellent component for the controlled and real time release of H_2S.

Light Triggered Uncaging of Hydrogen Sulfide (H_2S) with Real-Time Monitoring

pHP-Benz group always remain in center of attraction because its undoubtedly enhanced fluorescence (due to ESIPT process), real time monitoring by invasive fluorescence color change ability and biocompatible photoproduct production after photolysis [9]. These exciting features encourage Y venkaresh et. al[41] to use pHP-Benz group as a H_2S donor (2,2'-thiobis(1- (3-(benzo[d]thiazol-2-yl)-4 hydroxyphenyl)ethanone)) for controlled release of H_2S in visible light (Figure 7a). They have synthesized the donor molecule, at first by treating Na_2S with brominated pHP group and then, by treatment with 2-aminothiol. Their synthesized donor molecule has potentiality to keep the photophysical properties of the pHP-Benz intact. The photorelease ability was examined by passing the visible light source through the solution containing 1x 10^{-4} M H_2S donor molecule in acetonitrile/PBS buffer (7:3). The 125 W medium pressure mercury lamp with incident intensity (Io) of 2.886×1016 quanta s^{-1} was used as a visible light source and to get the $\lambda \geq 410$ nm 1M $NaNO_2$ solution was used as a UV cut-off filter.

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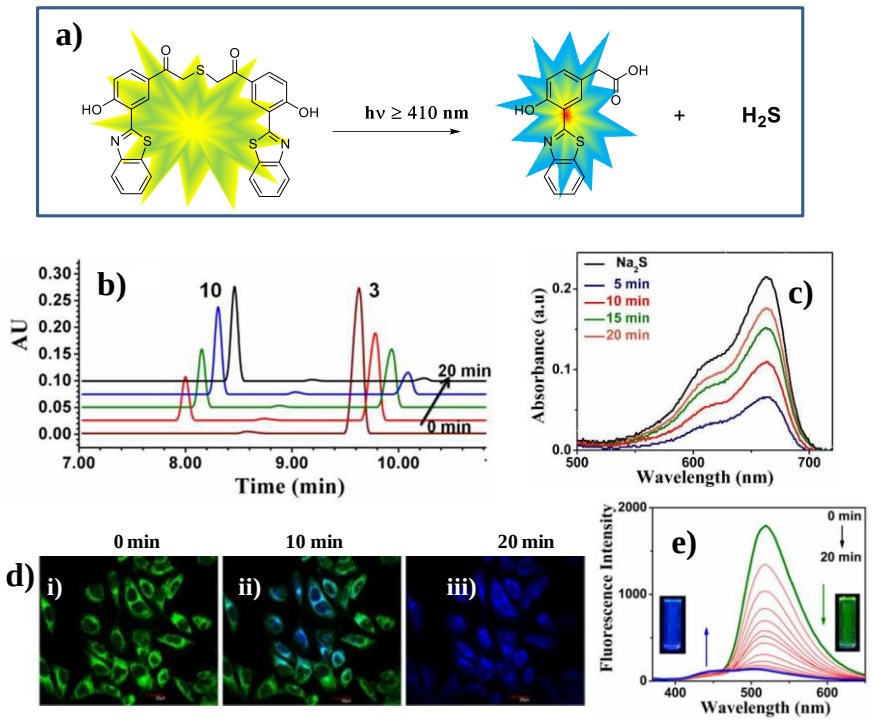

Figure 7: a) H2S release from pHP-Benz group (Schematic representation); b) RP-HPLC chromatogram for the release of H2S from pHP-Benz group; c) Methylene blue assay to check the H_2S release; d) fluorescence images of H2S donor moleculetreated Hela cell line at different visible light irradiation time (i) 0 min, (ii) 10 min and, (iii) 20 min; e) real time monitoring study (fluorescence color change from green to blue). (b-e) is reprinted with permission from ref. Copyright 2018: Royal Society of Chemistry.

Total irradiation time was 20 min and gradual disappearance of peak at tR=9.62 min in RP-HPLC chromatogram proved the efficient photodecomposition of H_2S donor upon light irradiation (Figure 7b). Methylene blue assay has been performed to check the H_2S release ability (Figure 7c). For this study, 100 µM solution of H_2S conjugated synthesized molecule in pH 7.4 acetonitrile/PBS buffer (3:7) was irradiation at λ ≥ 410 nm and absorption spectra was recorded at definite time interval. The gradual increment of absorption band at 663 nm proved the production of H_2S upon photo irradiation and the calculated time-dependent H_2S production rate constant was 1.32×10^7 s^{-1}. They also have used H_2S-sensitive electrode to examine the production of H_2S for individual system and they revealed that light is the reasons of H_2S production from H_2S donor molecule. During photolysis pHP-Benz group showed its unique property of fluorescence color change from green to blue (due to disruption of conjugation from the phenolic hydroxy group to the carbonyl group) and production of biocompatible photoproduct pHP-Benz-COOH (via photo favorskii mechanism) (Figure 7e). Cervical cancer HeLa cellline was used and incubated with H_2S donor molecule for 6 hours for cellular study (Figure 7d). The incubated cells showed green fluorescence before irradiation and showed blue fluorescence on post irradiation for 20 min. The blue fluorescence indicated the complete photolysis of H_2S donor to pHP-Benz-COOH and H_2S and further coumarin–hemicyanine fluorescence dye was used to track the intracellular H_2S level. In a word, 2,2'-thiobis(1- (3-(benzo[d]thiazol-2-yl)-4 hydroxyphenyl)ethanone) was a wonderful H_2S donor molecule had an ability to absorb visible light, had ability to show real time monitoring and release H_2S in a controlled manner using light as a reagent.

A two-photon responsive hydroxyphenylquinazolinone (HPQ)-based fluorescent organic nanoprodrug for H_2S release against oxidative stress

Chowdhury et. al [42] very recently reported that their synthesized hydroxyphenylquinazolinone (HPQ)-based fluorescent probe can release H_2S in a controlled way upon photo-irradiation in NIR region (Figure 8a). This is the step upgradation of H_2S released photoresponsive system from the previously reported pHP-TPE and pHP-Benz group. Newly synthesized HPQ-based single fluorescent component can form nanoparticles and can release H_2S in phototherapeutic window.

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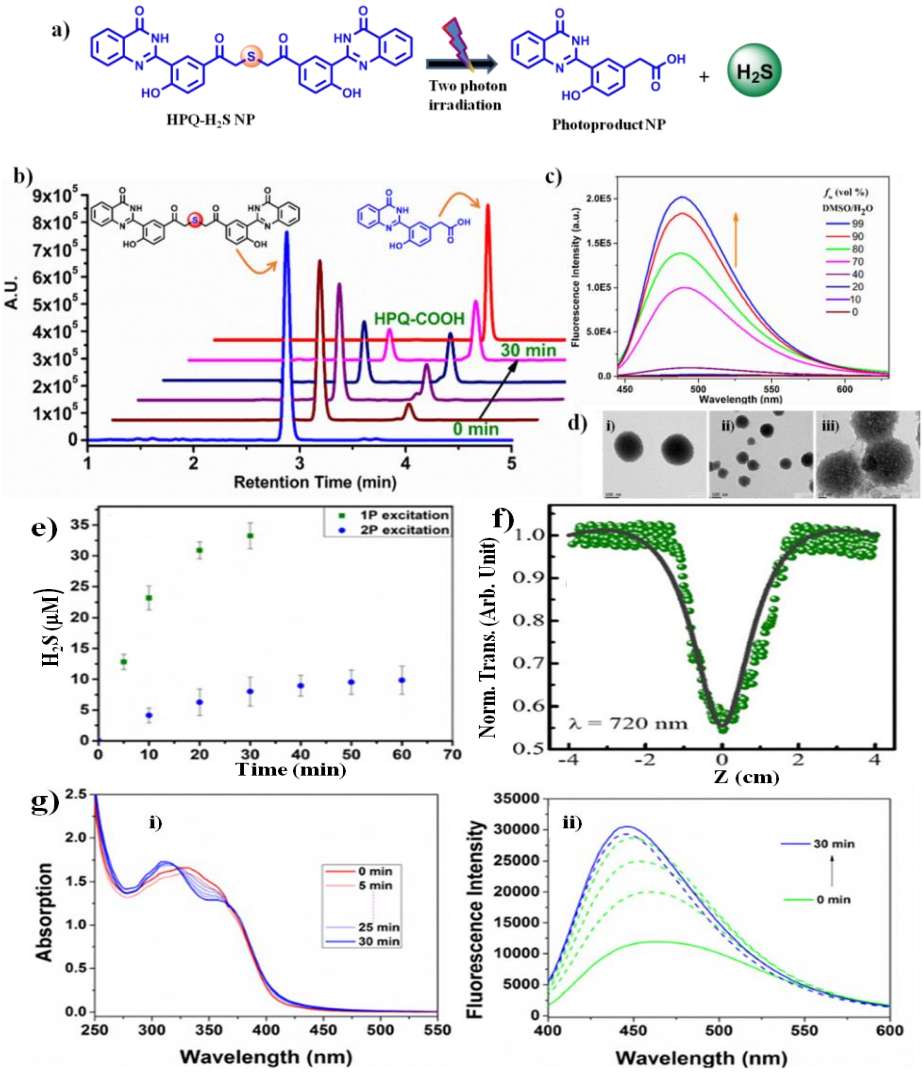

Figure 8: a) Schematic representation of two photon based H_2S release from HPQ-H_2S nanoparticles; b) RP-HPLC chromatogram for the release of H_2S release from HPQ-H_2S nanoparticles where peak at retention time 3 min represent the reactant HPQ-H_2S nanoparticles and 4 min peak corresponds to photoproduct; c) Aggregation based fluorescence enhancement study which further supports AIE process; d) TEM images (i) HPQ-H_2S nanoparticles, (ii) photoproduct of HPQ-H_2S nanoparticles, (iii) condition of nanoparticles after complete irradiation for 30 min; e) amounting the released H_2S for one and two photon irradiation with respect to time of irradiation; f) OA (OPEN APETURE) Z-scan traces for HPQ-H_2S nanoparticles ; g) (i) UV-absorption and (ii) Emission spectra of photolysis solution in different time intervals (0-30 min). (b-g) is reprinted with permission from ref. Copyright 2023: Royal Society of Chemistry.

HPQ-H_2S has been synthesized first by thio-ether bond formation using $Na_2S,9H_2O$ in starting materials then by condensation with 2-aminothiophenol. HPQ-H_2S conjugates shows AIE effect and examined by the study of rapid fluorescence intensity increment with incremental addition of water fraction (fw = 0-99%) and result also support the nanoparticle formation (Figure 8c-d). The nanoparticle formation again rechecks by following "simple reprecepitation technique" at 10^{-5} (M) concentration. The nanoprodrug was globular in shape and 95 nm of diameter. The photoluminescence spectra revealed that the HPQ-H_2S nanoprodrug has absorption upto visible region (upto 410 nm) and emission maxima at 480 nm with cyano colour fluorescence . During photorelease of H_2S from HPQ-H_2S conjugate, it follows the trend of parent pHP conjugate which is disruption of conjugation via photofavorskii mechanism. This phenomenon helps HPQ-H_2S conjugate to response in real time monitoring ability. Before in vitro studies, they have checked the stability of nano-conjugate and obtain only 12% of degradation in nanoconjugate after continuous 7 days of incubation. Standard methylene blue assay technique was utilized to quantify the released H_2S gas and ~ 35 μM of H_2S was released upon 30 min of irradiation at $\lambda \geq 365$ nm. Then single

beam open aperture (OA) Z-scan technique was used to investigate the two-photon absorption cross-section and it was 283 GM at 720 nm (Figure 8f). HPQ-H_2S nanoconjugate was irradiated at 720 nm for 1 h and the obtained H_2S release rate was 7.1×10^{-3} s^{-1} i.e, 25% decomposition (Figure e). Chowdhury et al describe the old pattern of photorelease mechanism of HPQ-H_2S conjugate as like parent pHP-Benz moiety. Cellular fluorescence imaging study and MTT assay have been carried out in MDA-MB-468 cell line and they did not observe any kind of inhibition of cell proliferation by HPQ-H_2S nanoconjugate upto a concentration of 100 µM before and after photoirradiation. Cyano to blue fluorescence colour change also observed in cellular study before and after 30 min of irradiation. In a word, the synthesized two photon responsive NIR active HPQ-H_2S is excellent in treatment in oxidative stress related disease.

H_2S_2 release

Real-time monitoring of a photoactivated hydrogen persulfide donor for biological entities

Endogeneously produced hydrogen persulfide(H_2S_2), a key ingredient for sulfur-based redox signaling mechanism. Endogeneously generated hydrogen polysulfides (H2Sn, n≥2) and perthiols (RSnH, n≥2) shows higher efficacy than that of H_2S in the healing of oxidative stress [44-47]. Hence, chowdhuri et al[43] had synthesized photoactivated hydrogen persulfide donor taking pHP-Benz moiety (Figure 9a). The excellence of the molecule is that-

i) External source light is the only requirement for release of H_2S_2
ii) Before (green) and after (blue) two different fluorescence colour leads to real-time monitoring (Figure 9f)
iii) Photoproduct is biocompartible and water soluble

Hydrolytic stability of the compound was investigated in DMSO/PBS buffer taking 100 µM H_2S_2 donor molecule and result showed that the compound was quite stable. H_2S_2 –donor molecules showed excellent stability at pH = 7.4 (analysed by RP-HPLC).

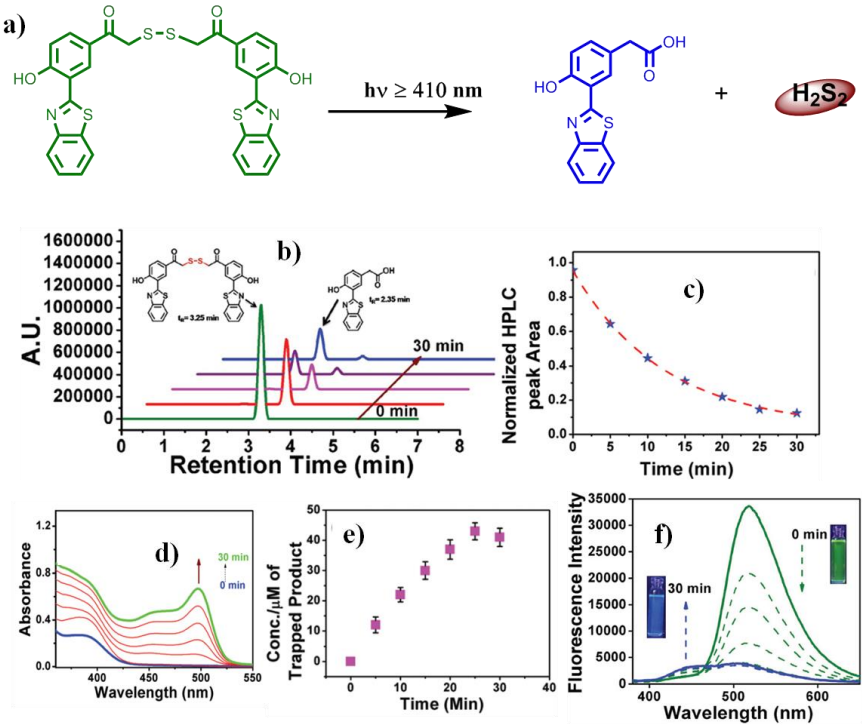

Figure 9: a) Sketching of H_2S_2 release from H_2S_2-donor molecule; b)RP-HPLC overlay chromatogram of H_2S_2 –donor molecule at 0-30 min time intervals; c)Exponential graph (HPLC peak area vs irradiation time) of disappearance of H_2S_2 donor molecule. The rate constant was 7.11 x 10-2.s-1. d) Recorded absorption spectra of collected photolysis fraction solution at different irradiation time; e) Quantification of released H_2S_2 at different irradiation time; f) Fluorescence spectral graph showing the real time monitoring ability of H_2S_2 donor molecule. (b-f) is reprinted with permission from ref. Copyright 2019: Royal Society of Chemistry.

To check the photochemical drug release ability H_2S_2-donor molecule (100 μM) was taken in PBS buffer and exposed to visible light with wavelength λ≥410 nm for 30 min. After that, the photo decomposed product was analysed by RP-HPLC. In HPLC they observed two peaks at retention time 3.25 min, 2.35 min correspond to H_2S_2-donor and photoproduct respectively (Figure 9b). To detect the release of H_2S_2, Fluorescein-based probe was used and quantification of released H_2S_2 was done from the HPLC peak area (Figure 9e). They have quantified that ~ 12 ± 3 μM H_2S gas was released from 100 μM of H_2S_2 donor molecule. Their proposed photorelease mechanism is same as the parent pHP-Benz molecule. MDA-MB-468 cancer cell was used for cellular uptake study and checking the real time monitoring ability of the H_2S_2 donor. The result showed that H_2S_2-donor treated cell were quite efficient in defending the oxidative stress than the untreated cell.

CONCLUSION

Controlled delivery of any biologically active substance has always remained on the top of the demand. When we can control the internal process like drug delivery by external stimulus like light, is added an extra flavor or advantages with the whole process. Researchers have carefully modified the chemical property of pHP group in such a that it can release very fast as always, and absorption of the group has been enhanced from UV to visible to NIR region. The pHP group showed biocompatibility in in-vitro analysis. In a word we can say, pHP photoremovable protecting group is very powerful and potential agent in the field of photochemistry and controlled delivery.

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