DOI:10.1021/jf1027763

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Fluorescent Caged Compounds of 2,4-Dichlorophenoxyacetic Acid (2,4-D): Photorelease Technology for Controlled Release of 2,4-D

Sanghamitra Atta, Avijit Jana, Rajakumar Ananthakirshnan, and Pradeep Singh Narayana Dhuleep*

Department of Chemistry, Indian Institute of Technology, Kharagpur 721302, India

A novel controlled-release formulation (CRF) of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was developed to reduce its negative environmental impacts by improving its herbicidal efficacy. The 2,4-D was chemically caged by coupling with photoremovable protecting groups (PRPGs) of coumarin derivatives. Photophysical studies of caged compounds showed that they all exhibited strong fluorescence properties. Controlled release of 2,4-D was achieved by irradiating the caged compounds using UV−vis light (310, 350, and 410 nm). The effect of various factors such as pH, solvent, and different substituents at the seventh position of coumarin moiety on the rate of photorelease was studied. The herbicidal activity of caged compounds and 4-(hydroxymethyl)-7-substituted coumarins was studied against *Vigna radiata*. The new formulation provided greater control over the release of 2,4-D by UV−vis light and also demonstrated the potential of the PRPGs not only to act as a delivery device but also to possess herbicidal activity after photorelease.

KEYWORDS: Caged compound; fluorescence; photoremovable protecting group; coumarin; 2,4-dichlorophenoxyacetic acid; controlled release

INTRODUCTION

Pesticides often have short effective lifetimes on target sites because they encounter various problems such as volatilization, leaching, and degradation. Enhancement of the pesticidal efficacy on target sites requires repeated pesticide applications, which is undesirable because of high cost, possible phytotoxicity, and, more importantly, certain pesticides are well-known as environmental pollutants (*1*). Hence, controlled-release formulations (CRFs) of pesticides have gained great interest because they allow usage of minimum amounts of pesticide for the same period of activity, thereby decreasing the risk to the environment (*2*).

CRFs developed so far are based on either physical encapsulation of active agents with polymeric materials, in which the polymer acts as a rate-controlling device or chemical combinations, in which the polymer acts as carrier for the agent. In the case of chemical combination, the active agent is chemically attached to a natural or synthetic polymer by a specific chemical bond, via either an ionic or covalent linkage. The release of the active agent is then primarily dependent on the rate of cleavage of the polymer−active agent bonds (*3*).

Recently, CRFs for pesticides based on chemical combination have received great interest because they offer safer, more efficient, and more economical crop protection (*4*). To date, controlled release of pesticides based on chemical combination is carried out by either hydrolytic or microbial cleavage of the chemical linkage between the pesticide and the polymeric backbone (*5*). To the best of our knowledge, there has been no report on cleavage of the polymer−pesticide bonds by light. This prompted us to develop a new type of formulation based on chemical combination utilizing light for the controlled release of pesticides. Furthermore, we were also interested in designing a delivery device that not only acts as a carrier for the pesticides but also possesses pesticidal activity after it releases the pesticides.

In recent years, use of photoremovable protecting groups (PRPGs) for the release of active molecules has become the subject of expanding interest because it allows both spatial and temporal control over the release (*6*). PRPGs are functional groups which are used to cage an active molecule in such a way that the activity of the molecule is masked. Later, exposure to light releases the protecting group, restoring functionality to the molecule. On the basis of the above strategy, several bioactive molecules including nucleic acids (*7*), amino acids, enzyme substrates, and catalysts of biochemical reactions (*8*) were chemically caged using PRPGs and released on irradiation by using a UV−visible light source. Recently, Gudmundsdottir's group also demonstrated the controlled release of fragrances under UV light over an extended period of time by chemically caging volatile alcohols such as geraniol using a PRPG (*9*).

Among the PRPGs, some of the groups are fluorescent and have greater advantage over nonfluorescent protecting groups because they not only release molecules of interest at desired locations for a specific period of time but also allow us to visualize, quantify, and follow the spatial distribution, localization, and depletion of the caged compounds by using techniques far more sensitive than UV (*10*−*14*). Consequently, this encouraged us to use

*Corresponding author [phone (+) 91-3222-282324; fax (+) 91-3222-282252; e-mail ndpradeep@chem.iitkgp.ernet.in].

 Published on Web 10/25/2010

fluorescent PRPG as a delivery device for controlled release of pesticides because in addition to its controlled release, it will also enable detection of caged pesticide residues at low level.

Herein, we report a new controlled-release formulation in which 2,4-dichlorophenoxyacetic acid (2,4-D) is chemically caged by fluorescent PRPGs of coumarin derivatives and later uncaged using UV−vis light. For the present study, we selected 2,4-D because it is a widely used herbicide and, more importantly, because it has a high leaching potential, which poses a threat to surface and groundwater contamination (*15*, *16*). In the case of PRPGs, coumarin-based protecting groups were preferred for caging 2,4-D due to their unique properties, such as (i) they allow the rate of photorelease, absorption maxima, and the solubility of their caged compounds to be altered by having suitable substituent at 6/7-position of their moiety (*12*); (ii) most of the coumarin derivatives exhibit strong fluorescence properties (*17*); and (iii) more importantly, compounds having a coumarin skeleton are known to possess pesticidal activity (*18*).

MATERIALS AND METHODS

Chemicals. Ethyl acetoacetate, 2,4-D, resorcinol, molecular bromine, formaldehyde, acetaldehyde, 2-[4-(2-hydroxyethyl)-1-piperazine]ethanesulfonic acid (HEPES) buffer, acetyl chloride, acetonitrile, and methanol were purchased from Merck. Dimethyl sulfate and ethyl chloroformate were purchased from Spectrochem Pvt. Ltd. *m*-Amino phenols and DMSO-d_6 were purchased from Sigma-Aldrich. All of the chemicals received were of analytical grade and used without further purification. Double-distilled water was used in this experiment. All of the stock solutions were kept in the refrigerator prior to use.

Instrumentation. ^{1}H NMR (400 MHz) spectra were recorded on a Bruker-AC 400 MHz spectrometer. Chemical shifts are reported in parts per million from tetramethylsilane with the solvent resonance as the internal standard (deuterodimethyl sulfoxide, 2.54 ppm). Data are reported as follows: chemical shifts, multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet), coupling constant (hertz). ^{13}C NMR (100 MHz) spectra were recorded on a Bruker-AC 400 MHz spectrometer with complete proton decoupling. Chemical shifts are reported in parts per million from tetramethylsilane with the solvent resonance as the internal standard (deuterodimethyl sulfoxide, 40.45 ppm). Chromatographic purification was done with 60−120 mesh silica gel (Merck). For reaction monitoring, precoated silica gel 60 F254 TLC sheets (Merck) were used. UV−vis absorption spectra were recorded on a Shimadzu UV-2450 UV−vis spectrophotometer, and fluorescence emission spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer. FT-IR spectra were recorded on a Perkin-Elmer RXI spectrometer. High-resolution mass spectra were recorded using a Qtof Micro YA263 mass spectrometer. RP-HPLC was performed using Waters 2489 liquid chromatography on a C_{18} column (4.6 mm × 250 mm) with a UV−vis detector. Photolysis of all the caged compounds was carried out using a 125 W medium-pressure mercury lamp supplied by SAIC (India).

Methods. *General Procedure for the Synthesis of the Caged Compounds* (***2a−g***). 4-(Bromomethyl)-7-substituted coumarin **1a−g** (1 equiv) was dissolved in dry *N*,*N* dimethylformamide (DMF) (2 mL). To the solution were added potassium iodide (2 equiv), potassium carbonate (2 equiv), and 2,4-D (1 equiv). The reaction mixture was stirred at 55 °C for 3 h. After completion of the reaction, solvent was removed under vacuum. To the crude residue was added ethyl acetate (EtOAc), followed by washing with brine water. The organic layer was collected, dried over Na_2SO_4, and evaporated under vacuum to yield a reddish brown residue, which was further purified by column chromatography using eluant 20% EtOAc in *n*-hexane.

2a ((*2,4-Dichlorophenyl*)*acetic acid 7-hydroxy-2-oxo-2H-chromen-4-ylmethyl ester*)*:* cream-colored solid; mp, 206 °C; UV−vis (MeOH 80/HEPES 20), λ_{max} (ε $M^{-1}cm^{-1}$) 291 (0.8×10^4), 322 (1.4×10^4); FTIR (KBr), υ_{max} (cm^{-1}) 3210 (OH), 1758 (ester OCO), 1703 (lactone OCO); ^{1}H NMR (DMSO-d_6, 400 MHz), δ 10.61 (s, 1, OH), 7.57 (d, 1, J = 2.4, Ar), 7.54 (d, 1, J = 8, Ar), 7.32 (dd, 1, J = 2.4, 8, Ar), 7.17 (d, 1, J = 8, Ar), 6.78 (dd, 1, J = 2.4, 8, Ar), 6.72 (d, 1, J = 2.4, Ar), 6.27 (s, 1, OCOCH), 5.41 (s, 2, OCH_2CO), 5.14 (s, 2, OCH_2C); ^{13}C NMR (DMSO-d_6, 100 MHz), δ 168.1, 161.7, 160.4, 155.3, 152.4, 150.2, 129.8, 129.3, 128.2, 126.5, 126.3, 125.6, 122.7, 115.5, 113.4, 109.3, 108.6, 102.8, 65.7, 62.1; HRMS (ES+), m/z calcd for $C_{18}H_{12}O_6Cl_2$ $[M + Na]^+$, 416.9908; found, 417.0708.

2b ((*2,4-Dichlorophenyl*)*acetic acid 7-methoxy-2-oxo-2H-chromen-4-ylmethyl ester*)*:* yellow solid; mp, 172 °C; UV−vis (MeOH 80/HEPES 20), λ_{max} (ε M^{-1} cm^{-1}) 291 (0.6×10^4), 321 (1.4×10^4); FTIR (KBr), υ_{max} (cm^{-1}) 1755 (ester OCO), 1724 (lactone OCO); ^{1}H NMR (DMSO-d_6, 400 MHz), δ 7.62 (d, 1, J = 8, Ar), 7.57 (s, 1, Ar), 7.32 (dd, 1, J = 2.4, 8, Ar), 7.17 (d, 1, J = 8, Ar), 7.02 (s, 1, Ar), 6.92 (d, 1, J = 8, Ar), 6.35 (s, 1, OCOCH), 5.44 (s, 2, OCH_2CO), 5.14 (s, 2, OCH_2C), 3.84 (s, 3, OCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz), δ 168.1, 162.9, 160.3, 155.3, 152.4, 150.1, 129.8, 128.3, 126.2, 125.6, 122.7, 115.5, 112.6, 110.5, 109.6, 101.3, 65.7, 62.2, 56.3; HRMS (ES+), m/z calcd for $C_{19}H_{14}O_6Cl_2$ $[M + H]^+$, 409.0245; found, 409.0177.

2c ((*2,4-Dichlorophenyl*)*acetic acid 7-acetoxy-2-oxo-2H-chromen-4-ylmethyl ester*)*:* cream-colored solid; mp, 179 °C; UV−vis (MeOH 80/HEPES 20), λ_{max} (ε M^{-1} cm^{-1}) 280 (0.6×10^4), 314 (1.1×10^4); FTIR (KBr), υ_{max} (cm^{-1}) 1763 (ester OCO), 1717 (lactone OCO), 1616 ($OCOCH_3$); ^{1}H NMR (DMSO-d_6, 400 MHz), δ 7.77 (d, 2, J = 8, Ar), 7.58 (s, 1, Ar), 7.34 (s, 1, Ar), 7.30 (s, 1, Ar) 7.18 (d, 1, J = 8, Ar), 7.15 (s, 1, Ar), 6.53 (s, 1, OCOCH), 5.48 (s, 2, OCH_2CO), 5.15 (s, 2, OCH_2C), 2.06 (s, 3, $COCH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz), δ 169.1, 168.1, 159.7, 154.0, 153.4, 152.4, 149.7, 129.8, 128.3, 126.3, 125.6, 122.7, 119.0, 115.5, 115.0, 112.5, 110.7, 65.7, 62.1, 21.2; HRMS (ES+), m/z calcd for $C_{20}H_{14}O_7Cl_2$ $[M + H]^+$, 437.0194; found, 437.0757.

2d ((*2,4-Dichlorophenyl*)*acetic acid 2-oxo-7-propioxylamino-2H-chromen-4-ylmethyl ester*)*:* light yellow solid; mp, 183 °C; UV−vis (MeOH 80/HEPES 20), λ_{max} (ε M^{-1} cm^{-1}) 292 (1.0×10^4), 327 (2.1×10^4); FTIR (KBr), υ_{max} (cm^{-1}) 1734 (ester OCO), 1706 (lactone OCO); ^{1}H NMR (DMSO-d_6, 400 MHz), δ 10.17 (s, 1, NH), 7.63 (d, 1, J = 8, Ar), 7.58 (s, 1, Ar), 7.57 (s, 1, Ar), 7.36 (d, 1, J = 8, Ar), 7.32 (d, 1, J = 8, Ar), 7.17 (d, 1, J = 8, Ar), 6.37 (s, 1, OCOCH), 5.43 (s, 2, OCH_2CO), 5.14 (s, 2, OCH_2C), 4.15 (q, 2, J = 7.2, 14.0, OCH_2CH_3), 1.24 (t, 3, J = 7.2, OCH_2CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz), δ 168.1, 160.2, 154.3, 153.7, 152.4, 149.9, 143.5, 129.8, 128.3, 125.8, 125.6, 122.7, 115.5, 114.7, 111.6, 110.3, 104.8, 65.7, 62.1, 61.1, 14.7; HRMS (ES+), m/z calcd for $C_{21}H_{17}NO_7Cl_2$ $[M + H]^+$, 466.0460; found, 466.0448.

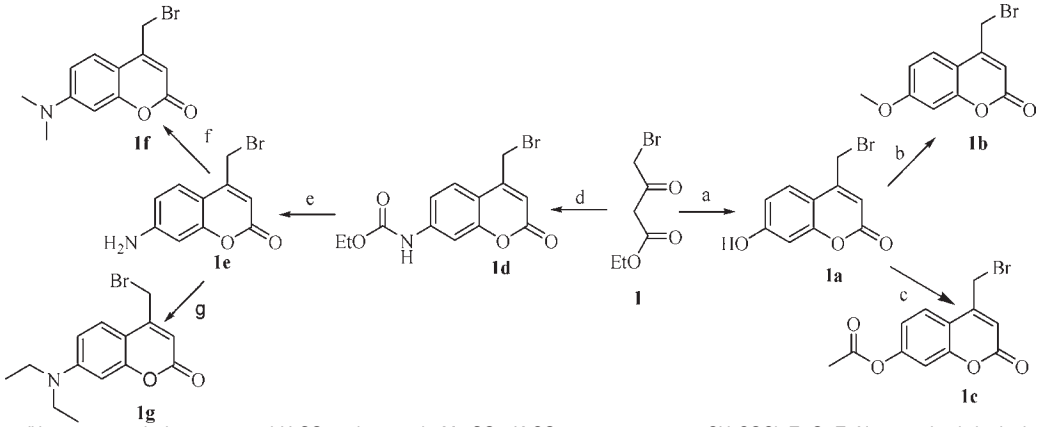
2e ((*2,4-Dichlorophenyl*)*acetic acid 7-amino-2-oxo-2H-chromen-4-ylmethyl ester*)*:* yellow solid; mp, 176 °C; UV−vis (MeOH 80/HEPES 20), λ_{max} (ε M^{-1} cm^{-1}) 291 (0.3×10^4), 356 (1.6×10^4); FTIR (KBr), υ_{max} (cm^{-1}) 1774 (ester OCO), 1701 (lactone OCO); ^{1}H NMR (DMSO-d_6, 400 MHz), δ 7.57 (d, 1, J = 2.4, Ar), 7.34 (d, 1, J = 8, Ar), 7.31 (dd, 1, J = 2.4, 8, Ar), 7.16 (d, 1, J = 8, Ar), 6.53 (d, 1, J = 8, Ar), 6.41 (s, 1, Ar), 6.19 (s, 2, NH_2), 6.04 (s, 1, OCOCH), 5.34 (s, 2, OCH_2CO), 5.13 (s, 2, OCH_2C); ^{13}C NMR (DMSO-d_6, 100 MHz), δ 168.1, 160.9, 156.0, 153.6, 152.5, 150.5, 129.8, 128.3, 125.9, 125.6, 122.7, 115.5, 111.7, 106.1, 105.4, 98.9, 65.7, 62.2; HRMS (ES+), m/z calcd for $C_{18}H_{13}NO_5Cl_2$ $[M + H]^+$, 394.0249; found, 394.0786.

2f ((*2,4-Dichlorophenyl*)*acetic acid 7-dimethylamino-2-oxo-2H-chromen-4-ylmethyl ester*)*:* yellow solid; mp, 186 °C; UV−vis (MeOH 80/HEPES 20), λ_{max} (ε M^{-1} cm^{-1}) 290 (0.4×10^4), 370 (2.0×10^4); FTIR (KBr), υ_{max} (cm^{-1}) 1774 (ester OCO), 1727 (lactone OCO); ^{1}H NMR (DMSO-d_6, 400 MHz), δ 7.57 (s, 1, Ar), 7.45 (d, 1, J = 8, Ar), 7.32 (d, 1, J = 8, Ar), 7.16 (d, 1, J = 8, Ar), 6,67 (d, 1, J = 8, Ar), 6.56 (s, 1, Ar), 6.10 (s, 1, OCOCH), 5.38 (s, 2, OCH_2CO), 5.13 (s, 2, OCH_2C), 3.00 (s, 6, NMe_2); ^{13}C NMR (DMSO-d_6, 100 MHz), δ 168.1, 160.9, 155.7, 153.2, 152.5, 150.3, 129.8, 128.3, 125.6, 122.7, 115.5, 109.5, 106.2, 106.0, 97.9, 79.5, 65.7, 62.2, 29.4; HRMS (ES+), m/z calcd for $C_{20}H_{17}NO_5Cl_2$ $[M + H]^+$, 422.0562; found, 422.0471.

2g ((*2,4-Dichlorophenyl*)*acetic acid 7-diethylamino-2-oxo-2H-chromen-4-ylmethyl ester*)*:* yellow solid; mp, 175 °C; UV−vis (MeOH 80/HEPES 20), λ_{max} (ε M^{-1} cm^{-1}) 291 (0.4×10^4), 381 (2.1×10^4); FTIR (KBr), υ_{max} (cm^{-1}) 1763 (ester OCO), 1720 (lactone OCO); ^{1}H NMR (DMSO-d_6, 400 MHz), δ 7.56 (d, 1, J = 2, Ar), 7.41 (d, 1, J = 8, Ar), 7.31 (dd, 1, J = 2, 8, Ar), 7.16 (d, 1, J = 8, Ar), 6.63 (d, 2, J = 8, Ar), 6.51 (s, 1, Ar), 6.07 (s, 1, OCOCH), 5.36 (s, 2, OCH_2CO), 5.13 (s, 2, OCH_2C), 3.39 (q, 2H, J = 3.2, 6.4, NEt_2), 1.11 (t, 3H, J = 6.4, NEt_2); ^{13}C NMR (DMSO-d_6, 100 MHz), δ 168.1, 160.9, 156.1, 152.5, 152.7, 150.2, 129.8, 128.2, 125.8, 125.6, 122.7, 115.5, 109.0, 108.0, 105.7, 97.2, 65.7, 62.2, 44.3, 14.4; HRMS (ES+), m/z calcd for $C_{22}H_{21}NO_5Cl_2$ $[M + H]^+$, 450.0875; found, 450.0871.

Photophysical Properties of Caged Compounds of 2,4-D (2a−g). The UV/vis absorption and emission spectra of degassed 3×10^{-5} M solution of caged compounds (**2a−g**) in MeOH:HEPES (80:20) were

Scheme 1. Synthesis of PRPG of Coumarin Derivatives (**1a**−**g**)[a]



[a] Reagents and conditions: a, resorcinol, concentrated H_2SO_4, 8 h, 50%; b, Me_2SO_4, K_2CO_3, acetone, 88%; c, CH_3COCl, Et_2O, Et_3N, 92%; d, ethyl 3-hydroxyphenyl carbamate, 70% H_2SO_4, 4 h, 82%; e, (1:1) concentrated H_2SO_4−glacial acetic acid, 4 h reflux, 78%; f, $NaBH_4$, THF, formaldehyde, 0 °C, 84%; g, $NaBH_4$, THF, acetaldehyde, 0 °C. 83%.

recorded. The Stokes' shift has been calculated from the difference in the absorption and the emission maxima of the caged compounds. Fluorescence quantum yield of the caged compounds was calculated using the eq 1.

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

where the subscripts CG and ST denote caged compound and standard, respectively. Quinine sulfate in 0.1 (N) H_2SO_4 solutions was taken as standard (*19*). 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.

Deprotection Photolysis of Caged Compounds of 2,4-D (2a−g). A solution of 10^{-5} M of the caged compound (**2a**−**g**) was prepared in MeOH/HEPES buffer (80:20). Half of the solution was kept in the dark and to the remaining half was passed nitrogen followed by irradiation at different UV wavelengths (310, 350, and 410 nm) individually, using a 125 W medium-pressure Hg lamp filtered by suitable filters with continuous stirring. At regular intervals of time, 20 μL aliquots were taken and analyzed by RP-HPLC using a mobile phase of acetonitrile/water (8:1), at a flow rate of 1 mL/min (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 was < 5% of the initial area.

On the basis of HPLC data for each caged compound, the natural logarithm of the concentration of caged compound (ln *C*) versus irradiation time was plotted. We observed a linear correlation for the disappearance of the caged compounds, which suggested a first-order reaction, obtained by linear least-squares methodology for a straight line. Photolysis half-life values of caged compounds were calculated using eq 2

$$t_{1/2} = \frac{0.693}{k_p} \quad (2)$$

where k_p is the first-order photolysis rate constant, obtained from the slope of the linear plot of ln *C* versus irradiation time. Furthermore, the quantum yield for the photolysis of caged compounds was calculated using eq 3

$$(\Phi_p)_{CG} = (\Phi_p)_{act.} \times \frac{(k_p)_{CG}}{(k_p)_{act.}} \times \frac{(F_{act.})}{(F_{CG})} \quad (3)$$

where the subscripts "CG" and "act." denote caged compound and actinometer, respectively. Potassium ferrioxalate was used as an actinometer (*20*). Φ_p is the photolysis quantum yield, k_p is the photolysis rate constant, and *F* is the fraction of light absorbed.

Preparative Photolysis. A solution of caged compound (**2a**−**g**) (0.05 mmol) in MeOH/HEPES (80:20) was irradiated using a 125 W medium-pressure Hg lamp filtered by suitable filters. The irradiation was monitored by TLC at regular intervals of time. After completion of photolysis, solvent MeOH/HEPES (80:20) was removed under vacuum, and the photoproducts (2,4-D and 4-(hydroxymethyl)-7-substituted coumarin) were isolated by column chromatography using increasing percentages of EtOAc in hexane as an eluant.

Laboratory Bioassay. Petri dishes of 9 cm diameter with a Whatman no. 1 filter paper were used for bioassay experiments. Each Petri dish was separately moistened with 10 mL of the tested compound (9.05×10^{-6} M). Control was similarly prepared, with the same amount of distilled water and free 2,4-D. Ten seeds of *Vigna radiata* (Moong Dal) were placed in each Petri dish. Each treatment was replicated three times. After 10 days of incubation (all of the Petri dishes were exposed to daylight for 30 min each day), shoot and root length were recorded. The data were analyzed by analysis of variance (ANOVA) followed by Duncan's multiple-range test to delineate the treatment means using SPSS computer software. The herbicidal activity was assessed as the inhibition rate in comparison with the distilled water.

RESULTS AND DISCUSSION

Synthesis of Photoremovable Protecting Groups (1a−g). We synthesized protecting groups of (coumarin-4yl)methyl type having different substituents at the 7-position such as 4-bromomethyl-7-hydroxychromen-2-one (**1a**) (*21*), 4-bromomethyl-7-methoxychromen-2-one (**1b**), acetic acid 4-bromomethyl-2-oxo-2*H*-chromen-7-yl ester (**1c**), (4-bromomethyl-2-oxo-2*H*-chromen-7-yl)carbamic acid ethyl ester (**1d**) (*22*), 7-amino-4-bromomethylchromen-2-one (**1e**), 4-bromomethyl-7-dimethylaminochromen-2-one (**1f**), and 4-bromomethyl-7-diethylaminochromen-2-one (**1g**) (*23*) as shown in **Scheme 1**.

Synthesis of Caged Compounds of 2,4-D (2a−g). The caging of 2,4-D using PRPG of coumarin derivatives was carried out by simple esterification as outlined in **Scheme 2**. The freshly prepared 4-(bromomethyl)-7-substituted coumarins (**1a**−**g**) were treated with 2,4-D in the presence of K_2CO_3/KI in dry DMF for 3 h at 55 °C, resulting in the formation of corresponding caged compounds (**2a**−**g**) in excellent yield (90−95%) as summarized in **Table 1**.

All of the caged compounds were characterized by IR, ^{1}H, ^{13}C NMR, and mass spectral analysis. The IR spectra of caged compounds (**2a**−**g**) showed bands in the range of 1755−1770 cm^{-1} due to the stretching vibration of the newly formed ester carbonyl group in addition to the carbonyl band of coumarin moiety at around 1710 cm^{-1}. ^{1}H NMR spectra showed signals corresponding to the ester methylene group (α-CH_2) at around δ 5.40 along with aromatic protons of 2,4-D at δ 6.65, 7.04, and 7.17 ppm. In addition, we also observed characteristic signals of the coumarin moiety at δ 6.10 (H-3, α,β-unsaturated alkene) and 7.16−7.57 (aromatic protons).

The confirmation of the presence of the newly formed ester group was further supported by the ^{13}C NMR spectra signal of the ester carbonyl at δ 168 apart from the carbonyl signal of the coumarin at δ 160.

Photophysical Properties of Caged Compounds of 2,4-D (2a−g). The photophysical properties of all the caged compounds were investigated. The absorption and emission maxima, molar absorptivities, Stokes' shift, and fluorescence quantum yield of the caged compounds (**2a−g**) are summarized in **Table 1**. The UV−vis absorption spectra (**Figure 1A**) clearly show caged compounds (**2a−g**) have two strong intense absorption bands centered in the region of λ = 321−381 nm and around λ = 220 nm. The long wavelength absorption band corresponds to coumarin chromophore (*10*) and the short wavelength is due to 2,4-D (*24*). As anticipated, different substituents at the 7-position on the coumarin moiety have great influence on the longer absorption wavelength band of the caged compounds (**Table 1**). For example, caged compounds **2f** and **2g** with an electron-donating dialkylamino substituent at the 7-position of coumarin showed a significant red shift of absorption maxima toward the visible region combined with increasing extinction coefficient (**Figure 1A**).

Furthermore, to understand the fluorescence properties of the caged compounds, the emission spectra (**Figure 1B**) were recorded by exciting the caged compounds (**2a−g**) at their corresponding absorption maxima in MeOH/HEPES (80:20). The caged compounds exhibited strong fluorescence with maximum emission wavelengths between 400 and 483 nm, and the magnitude of the Stokes' shift of all the caged compounds varies between 79 and 169 nm. Furthermore, the caged compounds also showed moderate fluorescence quantum yield ($0.59 < \Phi_f < 0.03$).

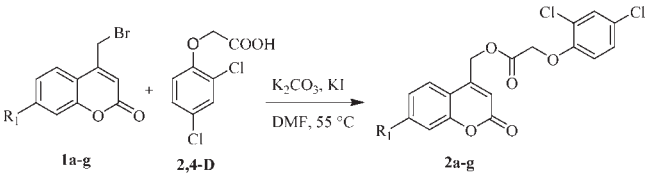
The photophysical studies revealed that caging of nonfluorescent 2,4-D by PRPG of coumarin derivatives showed strong fluorescence, large Stokes' shift, and good fluorescence quantum yield. Hence, the above method could be useful to detect caged pesticide residues at low level using a sensitive fluorescence technique.

Photolysis of Caged Compounds of 2,4-D (2a−g). Irradiation of caged compounds (**2a−g**) in MeOH/HEPES (80:20) buffer at different wavelengths (310, 350, and 410 nm) resulted in controlled release of 2,4-D (**Table 2**). In each case, the photolysis was stopped when conversion reached at least 95% (as indicated by HPLC). For all of the caged compounds mentioned in **Table 2**, the photolysis products (2,4-D and 4-(hydroxymethyl)-7-substituted coumarins) were confirmed by isolating and matching their ^{1}H NMR spectra to those of authentic samples.

As a representative example we have shown in **Figure 2A** the HPLC of the photolysis of caged compound **2a** at regular intervals of time. The HPLC chart shows that as the irradiation time increases we can observe a gradual decrease of the peak at R_t = 16.20 min, indicating the photocleavage of the caged compound **2a**. On the other hand, we also note a gradual increase of two new peaks at R_t = 3.14 and 2.42 min, corresponding to released 2,4-D and 4-(hydroxymethyl)-7-hydroxycoumarin, respectively. Furthermore, we also monitored the course of photorelease of caged compound **2g** using fluorescence (**Figure 2B**) and UV−vis (Supporting Information Figures S1 and S2) spectroscopy.

Similarly to previously discussed coumarinyl methyl caged compounds (*10*), the mechanism of the photocleavage of the caged compounds of 2,4-D involves initial heterolysis of the C−O ester bond (photo-S_N1) to produce an ion pair of coumarinyl methyl carbocation and a carboxylate anion of 2,4-D (**Scheme 3**). After ion pair separation in polar solvent, the methylenic carbocation is trapped by the solvent molecule to yield 4-(hydroxymethyl)-7-substituted coumarins (**3a−g**). On the other hand, the carboxylate anion abstracts a proton from the solvent to yield the corresponding 2,4-D.

Scheme 2. Synthesis of Caged Compounds of 2,4-D (**2a−g**)



R_1 = -OH (**1a**)
-OMe (**1b**)
-$OCOCH_3$ (**1c**)
-NHCOOEt (**1d**)
-NH_2 (**1e**)
-NMe_2 (**1f**)
-NEt_2 (**1g**)

2a = -OHCM-2,4-D
2b = -OMeCM-2,4-D
2c = -$OCOCH_3$CM-2,4-D
2d = -NHCOOEtCM-2,4-D
2e = -NH_2CM-2,4-D
2f = -NMe_2CM-2,4-D
2g = -NEt_2CM-2,4-D

Table 1. Synthetic Yield, UV−Vis, and Fluorescence Data for the Caged Compounds (**2a−g**)

		UV−vis		fluorescence		
caged compd	synthetic yield[a] (%)	λ_{max}[b] (nm)	ε[c] (10^4 M^{-1} cm^{-1})	λ_{max}[d] (nm)	Stokes' shift[e] (nm)	Φ_f[f]
2a	90	322	1.4	480	158	0.51
2b	93	321	1.4	400	79	0.13
2c	95	314	1.0	483	169	0.29
2d	94	327	2.1	414	87	0.41
2e	94	356	1.6	454	98	0.59
2f	91	370	2.0	476	106	0.18
2g	92	381	2.1	472	91	0.03

[a] Based on isolated yield. [b] Maximum absorption wavelength. [c] Molar absorption coefficient (M^{-1} cm^{-1}) at the maximum absorption wavelength. [d] Maximum emission wavelength. [e] Difference between maximum emission wavelength and maximum absorption wavelength. [f] Fluorescence quantum yield (error limit within ±5%) was calculated using quinine sulfate as standard (Φ_f = 0.54 in 0.1 N H_2SO_4).

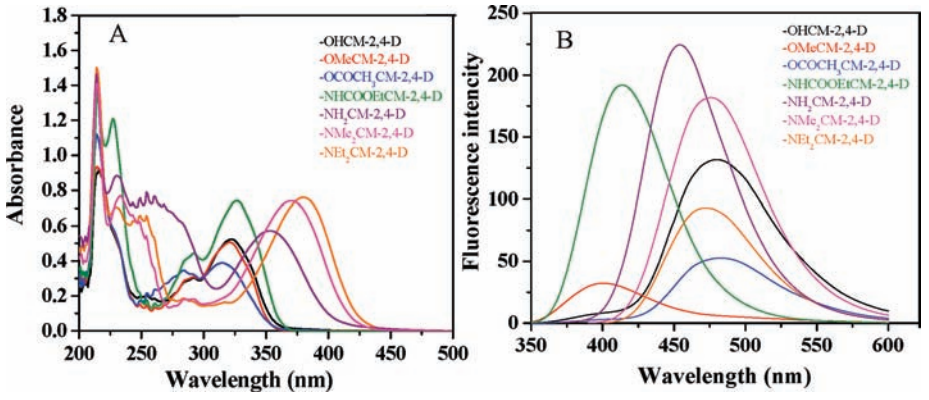


Figure 1. (**A**) UV−vis absorption spectra of the caged compounds (**2a−g**) in MeOH/HEPES (80:20) (3×10^{-5} M). (**B**) Corrected emission spectra of the caged compounds (**2a−g**) in MeOH/HEPES (80:20) (3×10^{-5} M).

Linear regression analysis of the natural logarithm of the concentration of caged compound (ln C) versus irradiation time at different irradiation wavelengths is shown in **Figure 3B**. The first-order photolysis rate constant (k_p) values of caged compounds at different wavelengths (**Table 2**) indicates that substituents at the 7-position of the coumarin moiety and the irradiation wavelength have pronounced effects on the release of 2,4-D.

Effect of Substituents at the 7-Position of the Coumarin Moiety on the Release of 2,4-D. The results from **Table 2** clearly show that different substituents at the 7-position of the coumarin moiety have great influence on the ability of the PRPG to release 2,4-D. At 310 nm, we note the efficiency of the caged compounds to release 2,4-D increases as the electron-donating character of the substituent at the 7-position of the coumarin moiety increases (caged compound **2b** with a stronger electron-donating −OMe group at the 7-position showed 2 times higher value of photolysis rate constant (k_p) compared to caged compound **2c** with a moderate electron-donating $-OCOCH_3$ substituent); this can be due to the stabilization of the intermediate coumarin$-CH_2^+$ by an electron donor group (*12*). Caged compounds **2f** and **2g**, with strong electron-donating dialkyl amino substituents, showed 3−5 times lesser rate constant (k_p) values compared to **2b**, because **2f** and **2g** have very weak absorption at around 310 nm. The above substituent effects on the 2,4-D release can also be noted from the quantum yield (Φ_p) results. **Figure 3A** shows the percentage of release of pesticide 2,4-D from different caged compounds (**2a**−**g**) after 20 h of irradiation at 310 nm, and the release ranges from 37 to 96%.

Table 2. Photolytic Data of Caged Compounds (**2a**−**g**) at Different Irradiation Wavelengths in MeOH/HEPES (80:20)

	310 nm			350 nm			410 nm		
caged compd	ε^a	$k_p{}^b$	$\Phi_p{}^c$	ε^a	$k_p{}^b$	$\Phi_p{}^c$	ε^a	$k_p{}^b$	$\Phi_p{}^c$
2a	0.8	4.470	0.018	0.3	0.866	0.012			
2b	0.8	2.887	0.011	0.3	0.715	0.011			
2c	0.8	1.377	0.005	0.1	0.381	0.009			
2d	1.1	2.166	0.007	0.8	0.488	0.004			
2e	0.8	0.985	0.004	1.6	0.533	0.002			
2f	0.3	0.806	0.003	1.6	0.205	0.001	0.9	0.059	0.001
2g	0.3	0.523	0.004	1.1	0.211	0.001	1.3	0.073	0.001

[a] Molar absorption coefficient (10^4 M^{-1} cm^{-1}) at the irradiation wavelength. [b] Rate constant (10^{-3} min^{-1}) under photolytic conditions., [c] Photochemical quantum yield (error limit within ±5%).

Effect of Irradiation Wavelength on the Release of 2,4-D. The influence of the irradiation wavelength on the extent of photocleavage of caged compounds (**2a**−**g**) in MeOH/HEPES buffer (80:20) is shown in **Table 3**. The time required for photocleavage of caged compounds increases as the irradiation wavelength increases. C−O bond dissociation energy is 86 kcal/mol, and as we increase the wavelength, the excitation energy decreases from 92 kcal/mol (310 nm) to 70 kcal/mol (410 nm). Hence, caged compound **2g** has a relatively short half-life ($t_{1/2}$ = 1325 min) at

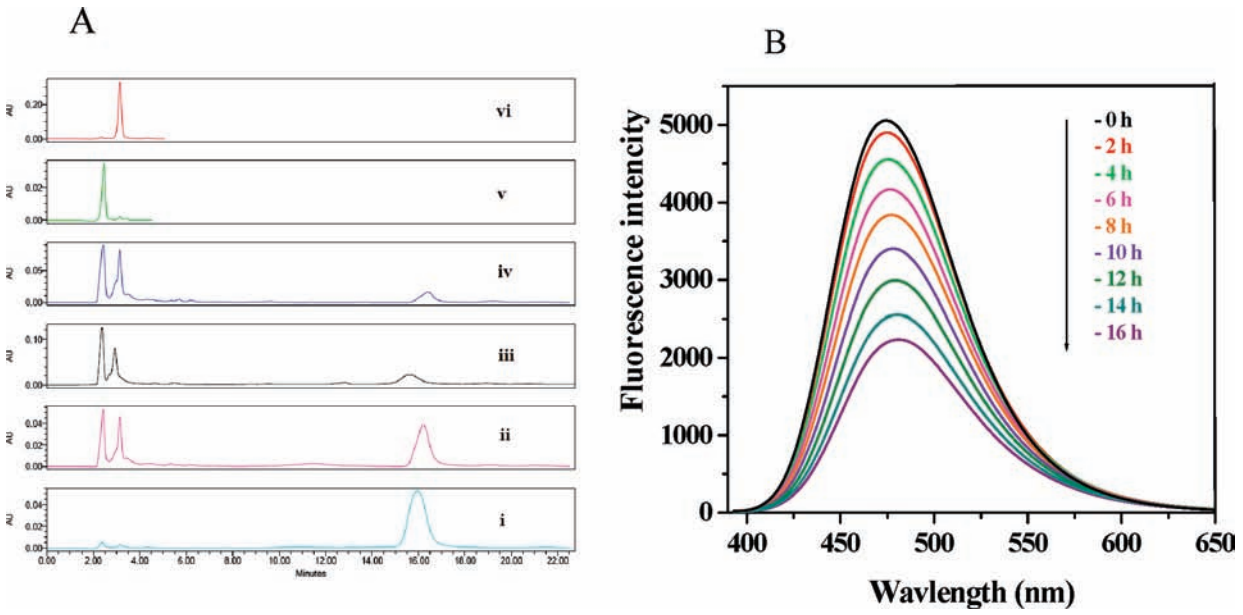
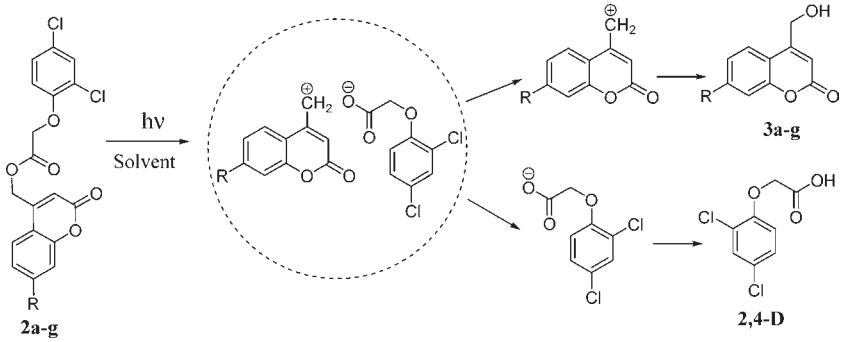


Figure 2. (**A**) HPLC data of photolysis of caged compound **2a** at regular intervals of time: (i) 0 h; (ii) 2 h; (iii) 4 h; (iv) 6 h; (v) 4-(hydroxymethyl)-7-hydroxycoumarin; (vi) std 2,4-D. (**B**). Corrected emission spectra of the caged pesticide **2g** at regular intervals of irradiation in MeOH/HEPES (80:20) (3×10^{-5} M).

Scheme 3. Mechanism of the Photolysis of Caged Compounds (**2a**−**g**) To Release 2,4-D



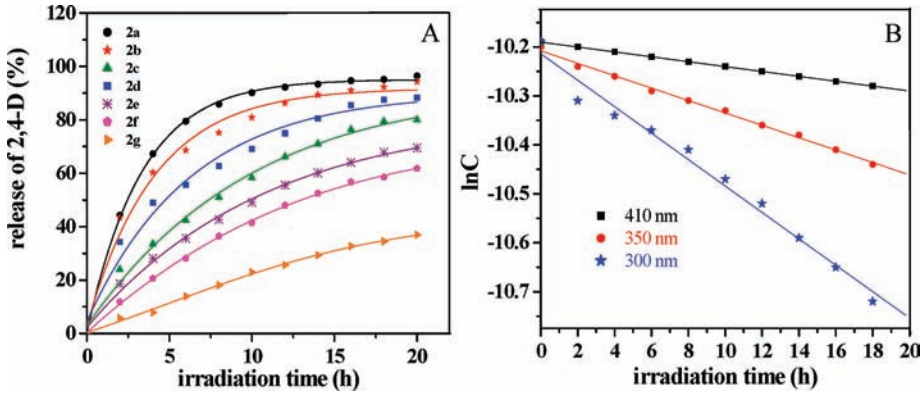


Figure 3. (**A**) Release of 2,4-D (%) versus irradiation time of the caged compounds (**2a**−**g**) in MeOH/HEPES (80:20) at 310 nm. (**B**) Plot of ln *C* versus irradiation time for the photolysis of **2g** in MeOH/HEPES (80:20) at 310, 350, and 410 nm.

Table 3. Half-Life ($t_{1/2}$) of Caged Compounds (**2a**−**g**) at Different Irradiation Wavelengths in MeOH/HEPES (80:20)

	$t_{1/2}$ (min)		
caged compd	310 nm	350 nm	410 nm
2a	155	800	-
2b	240	969	-
2c	503	1818	-
2d	319	1420	-
2e	703	1300	-
2f	859	3380	11588
2g	1325	3284	9493

Table 4. Photolytic Data of Compound **2g** at Different Irradiation Wavelengths in Different Solvent Systems

	310 nm			350 nm			410 nm		
solvent system	ε^a	$k_p{}^b$	$\Phi_p{}^c$	ε^a	$k_p{}^b$	$\Phi_p{}^c$	ε^a	$k_p{}^b$	$\Phi_p{}^c$
MeOH	0.2	0.966	0.016	1.2	0.415	0.002	0.8	0.100	0.002
MeOH/HEPES (80:20)	0.3	0.523	0.004	1.1	0.211	0.001	1.3	0.073	0.001
MeOH/HEPES (60:40)	0.3	0.558	0.004	0.9	0.307	0.002	1.2	0.083	0.001
MeOH/HEPES (50:50)	0.3	0.567	0.006	0.8	0.374	0.003	1.4	0.086	0.001
MeOH/HEPES (40:60)	0.4	0.588	0.005	0.9	0.448	0.003	1.5	0.131	0.002
ACN/HEPES (80:20)	0.4	0.768	0.003	1.3	0.583	0.003	0.9	0.184	0.004
THF/water (50:50)	0.3	0.546	0.002	1.2	0.431	0.002	1.2	0.128	0.002
EtOH/water (1:99)	0.3	1.037	0.009	0.8	0.694	0.005	1.5	0.260	0.003

[a] Molar absorption coefficient (10^4 M^{-1} cm^{-1}) at the irradiation wavelength. [b] Rate constant (10^{-3} min^{-1}) under photolytic conditions. [c] Photochemical quantum yield (error limit within ±5%).

310 nm compared to that at 410 nm ($t_{1/2}$ = 9493 min) (**Table 3**). The incident photon flux (I_0) at 310, 350, and 410 nm is 1.25×10^{17}, 9.66×10^{16}, and 4.12×10^{16} photons s^{-1} cm^{-2}, respectively.

Effect of Solvent on the Release of 2,4-D. To understand the solvent effect on the rate of photorelease of 2,4-D, we carried out the photolysis of caged compound **2g** in solvents of different polarity and character such as aqueous mixtures of methanol, acetonitrile, and tetrahydrofuran using UV−vis light (310, 350, and 410 nm). The initial concentration of the caged compound **2g** in each case was taken as 10^{-5} M, the release of pesticide 2,4-D was determined at regular intervals using HPLC, and the results are included in **Table 4**.

As anticipated, the character of the solvent has shown a pronounced influence on the efficiency of photorelease of 2,4-D; the quantum yield for the photorelease of 2,4-D by **2g** in methanol and acetonitrile was found to be higher compared to that in tetrahydrofuran. This can be attributed due to (i) the formation of an ionic intermediate (coumarin−CH_2^+) in **Scheme 3** and (ii) the hydrogen bonding and polarity of the solvents (*12*). The above fact was further confirmed by the increase in the quantum yield of photorelease of 2,4-D as the percentage of aqueous buffer in methanol increases. We also carried out the photolysis of caged compound **2g** in a water/ethanol (99:1) mixture and found much higher quantum yield for the photorelease of 2,4-D compared to other solvent systems used for the study. **Figure 4A** shows the percentage of release of pesticide 2,4-D from **2g** in different solvents after 20 h of irradiation and the release ranges from 35 to 70%.

The stability of the caged compounds (**2a**−**g**) was evaluated by keeping them in the dark in aqueous solvents for a period of 30 days. We observed < 10% decomposition of the caged compounds by HPLC.

Effect of pH on the Release of 2,4-D. To assess the role of pH on the rate of release of 2,4-D, the solution containing caged compound (**2g**) at different initial pH values in the range of 3.5−10.5 was irradiated using 310 nm. The photocleavage kinetics at different pH values clearly indicates that the release of 2,4-D is much slower at near-neutral pH in comparison to both acidic and basic pH values (**Figure 4B**), because ester hydrolysis is shown to be accelerated by acid and base. Furthermore, the release of 2,4-D on irradiation of **2g** for 20 h varied from 37 to 86% at different initial pH conditions (**Figure 4B**).

Herbicidal Activity. Preliminary results on the shoot and root length inhibition of *V. radiata* by the caged compounds (**2a**−**g**) and 4-(hydroxymethyl)-7-substituted coumarins (**3a**−**g**) obtained from the laboratory bioassay experiments are shown in **Figure 5**. The results indicate a 75−87% reduction in the shoot length of *V. radiata* with controlled release of 2,4-D using caged compounds (**2a**−**g**), whereas free 2,4-D showed a 90% reduction in shoot length. Among caged compounds, compounds **2a**−**c** exhibited better shoot length inhibition compared to compounds **2e**−**g**, because caged compounds **2a**−**c** showed efficient photorelease of 2,4-D in comparison to compounds **2e**−**g** (**Table 2**). We also observed a similar trend in root length inhibition (78−90%) by the caged compounds. Interestingly, we found that photoproducts 4-(hydroxymethyl)-7-substituted coumarins (**3a**−**g**) also displayed inhibition of shoot length (36−62%) and root length (50−73%) of *V. radiata*. Among the photoproducts, 4-(hydroxymethyl)-7-hydroxycoumarin (**3a**) was found to have better inhibition activity against *V. radiata*.

The newly developed delivery device for the controlled release of 2,4-D based on PRPG of coumarin derivatives provides advantages such as (i) greater control over the release by having a suitable substituent at the 7-position of the coumarin moiety; (ii) hydrolytic stability to the pesticide; (iii) the fluorescence of the caged pesticide mitigating low-level detection problems; and (iv) finally, the possibility of value-adding them to the formulation due to their pesticidal activity. In the future we will report our

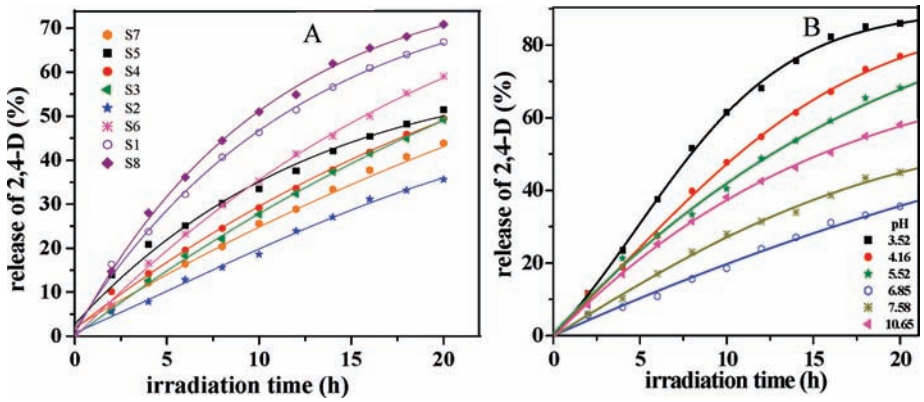


Figure 4. (**A**) Release of 2,4-D (%) on irradiation of **2g** at 310 nm in different solvent systems: (S1) methanol; (S2) methanol/HEPES (80:20); (S3) methanol/HEPES (60:40); (S4) methanol/HEPES (50:50); (S5) methanol/HEPES (40:60); (S6) acetonitrile/HEPES (80:20); (S7) THF/water (50:50); (S8) ethanol/water (1:99). (**B**) Release of 2,4-D (%) on irradiation of **2g** at 310 nm in different pH conditions.

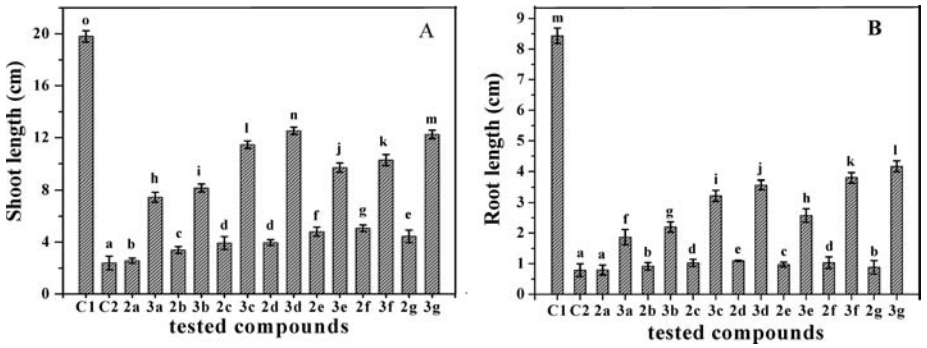


Figure 5. Effect of tested compounds on shoot and root length growth on *Vigna radiata*. Vertical bars show standard errors. Bars with different letters show significant difference ($P \leq 0.05$) as determined by Duncan's multiple-range test. C1 and C2 indicate controlled experiments using distilled water and free 2,4-D, respectively.

results on controlled release of 2,4-D in a soil medium using PRPG under sunlight.

ACKNOWLEDGMENT

We thank DST FIST for 400 MHz NMR and the Indian Association for the Cultivation of Science, Kolkata, for HRMS analysis. We thank Prof. S. Dasgupta and Prof. S. Nanda for their help in carrying out laboratory bioassay.

Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectra of compounds **2a**−**2g** and absorption spectrum of compound **2g** in MeOH/HEPES (60:40) solvent system under different irradiation times (Figure S1); absorption spectrum of compound **2g** in acetonitrile/HEPES (80:20) solvent system under different irradiation times (Figure S2); plot of ln *C* versus irradiation time of caged compound **2g** at 310 nm in different solvent systems and in different pH conditions (Figure S3); effect of tested compounds on shoot and root length growth on *Vigna radiata* (Table S1). This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Kirk, R. E. *Concise Encyclopedia of Chemical Technology*. Othmer, D. F., Ed.; Wiley: New York, 1985; Vol. 26, pp 652−656.

(2) Lewis, D. .H; Cowsar, D. R. Principles of controlled release pesticides. In *Controlled Release Pesticides*; Scher, H. B., Ed.; ACS Symposium Series 53; American Chemical Society: Washington, DC,1977; Chapter 1, pp 1−16.

(3) Kinawy, E. R.; Sherrington, D, C.; Akelah, A. Controlled release of agrochemical molecules chemically bound to polymers. *Eur. Polym. J.* **1992**, *28*, 841−862.

(4) Tai, L.; Liu, D.; Shen, Y.; Si, N. Biologically active polymer: controlled-release formulations based on Hymexazol. *Polym. Int.* **2002**, *51*, 1361−1365.

(5) Bhattacharya, S.; Sanyal, S. K.; Mukherjea, R. N. Controlled-release polymeric herbicide formulations with Pendent 2,4-dichlorophenoxyacetic acid. *Ind. Eng. Chem. Prod. Res. Dev.* **1986**, *25*, 585−589.

(6) Lee, H. M.; Larson, D. R.; Lawrence, D. S. Illuminating the chemistry of life: design, synthesis, and applications of "caged" and related photoresponsive compounds. *ACS Chem. Biol.* **2009**, *4*, 409−427.

(7) Folini, M.; Berg, K.; Millo, E.; Villa, R.; Prasmickaite, L.; Daidone, M. G.; Benatti, U.; Zaffaroni, N. Photochemical internalization of a peptide nucleic acid targeting the catalytic subunit of human telomerase. *Cancer Res.* **2003**, *63*, 3490−3494.

(8) Bochet, C. G. Photolabile protecting groups and linkers. *J. Chem. Soc., Perkin Trans. 1* **2002**, 125−142.

(9) Pika, J.; Konosonoks, A.; Robinson, R. M.; Singh, P. N. D.; Gudmundsdottir, A. D. Photoenolization as a means to release alcohols. *J. Org. Chem.* **2003**, *68*, 1964−1972.

(10) Schada, B.; Hagen, V.; Schmidt, R.; Herbrich, R.; Krause, E.; Eckardt, T.; Bendig, J. Deactivation behavior and excited-state properties of (coumarin-4-yl)methyl derivatives. 1. Photocleavage of (7-methoxycoumarin-4-yl)methyl-caged acids with fluorescence enhancement. *J. Org. Chem.* **1999**, *64*, 9109−9117.

(11) Hagen, V.; Frings, S.; Bendig, J.; Lorenz, D.; Wiesner, B.; Kaupp, U. B. Fluorescence spectroscopic quantification of the release of cyclic nucleotides from photocleavable [bis(carboxymethoxy)coumarin-4-yl]methyl esters inside cells. *Angew. Chem., Int. Ed.* **2002**, *41*, 3625−3628.

(12) Eckardt, T.; Hagen, V.; Schade, B.; Schmidt, R.; Schweitzer, C.; Bendig, J. Deactivation behavior and excited-state properties of (coumarin-4-yl)methyl derivatives. 2. Photocleavage of selected

(coumarin-4-yl)methyl-caged adenosin cyclic 3, 5-monophosphates with fluorescence enhancement. *J. Org. Chem.* **2002**, *67*, 703−710.
(13) Politz, J. C. Use of caged fluorochromes to track macromolecular movement in living cells. *Trends Cell Biol.* **1999**, *9*, 284−287.
(14) McGall, G. H.; Barone, A. D.; Diggelmann, M.; Fodor, S. P. A.; Gentalen, E.; Ngo, N. The efficiency of light-directed synthesis of DNA arrays on glass substrates. *J. Am. Chem. Soc.* **1997**, *119*, 5081−5090.
(15) Buser, H. R.; Muller, M. D. Occurrence and transformation reaction of chiral and achiral phenoxyalkanoic acid herbicides in lakes and rivers in Switzerland. *Environ. Sci. Technol.* **1998**, *32*, 626−633.
(16) Hyun, S.; Lee, L. S. Quantifying the contribution of different sorption mechanisms for 2,4-dichlorophenoxyacetic acid sorption by several variable-charge soils. *Environ. Sci. Technol.* **2005**, *39*, 2522−2528.
(17) Park, S. Y.; Ebihara, M.; Kubota, Y.; Funabiki, K.; Matsui, M. The relationship between solid state fluorescence intensity and molecular packing of coumarin dyes. *Dyes Pigm.* **2009**, *82*, 258−267.
(18) Alvarado, S. I.; Marc, P. A.; Dahle, B. J.; Reilly-Horch, E. 4-Phenoxycoumarins as herbicidal agents. U.S. Patent 5,681,968, Oct 28, 1997.
(19) Demas, J. N.; Crosby, G. A. The measurement of photoluminescence quantum yields. A review. *J. Phys. Chem.* **1971**, *75*, 991−1024.
(20) Kuhn, H. J.; Braslavsky, S. E.; Schmidt, R. Chemical actinometry. *Pure Appl. Chem.* **1989**, *61*, 187−210.
(21) Zhou, Z.; Fahrni, C. J. A fluorogenic probe for the copper(I)-catalyzed azide-alkyne ligation reaction: modulation of the fluorescence emission via $^{3}(n, \pi^*)-^{1}(\pi, \pi^*)$ inversion. *J. Am. Chem. Soc.* **2004**, *126*, 8862−8863.
(22) Atkins, R. L.; Bliss, D. E. Substituted coumarins and azacoumarins. Synthesis and fluorescent properties. *J. Org. Chem.* **1978**, *43*, 1975−1980.
(23) Ito, T.; Rokita, S. E. Reductive electron injection into duplex DNA by aromatic amines. *J. Am. Chem. Soc.* **2004**, *126*, 15552−15559.
(24) Kundu, S.; Pal, A.; Dikshit, A. K. UV induced degradation of herbicide 2,4-D: kinetics, mechanism and effect of various conditions on the degradation. *Sep. Purif. Technol.* **2005**, *44*, 121−129.

Received for review July 19, 2010. Revised manuscript received October 4, 2010. Accepted October 06, 2010. We thank DST (SERC Fast Track Scheme) for financial support. S.A. is grateful to UGC (India) and A.J. is thankful to CSIR (India) for their research fellowships.