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Development of first photoresponsive prodrug of paclitaxel

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Abstract—A prodrug of paclitaxel which has a coumarin derivative conjugated to the amino acid moiety of isotaxel (*O*-acyl isoform of paclitaxel) has been synthesized. The prodrug was selectively converted to isotaxel by visible light irradiation (430 nm) with the cleavage of coumarin. Finally, paclitaxel was released by subsequent spontaneous O–N intramolecular acyl migration.

A large number of anticancer agents have been developed in recent decades. However, these agents have very little or no specificity for the target tumor tissues which leads to systemic toxicity. Among them, paclitaxel (**1**, Taxol®) is considered to be one of the most important drugs in cancer chemotherapy. However, this agent also has low tumor selectivity.

To overcome this problem, prodrug strategy is specially promising.[1,2] A lot of prodrugs of paclitaxel have already been designed to specifically deliver them to the tumor tissues with a site-specific chemical delivery system. In addition, macromolecular prodrugs showed targetable properties due to enhanced permeability and retention (EPR) effect, and monoclonal antibodies (mAbs) were used as a vehicle to deliver **1** selectively to the tumor tissues. Some of them exhibited very promising properties; however, none of them is available in clinical use.[3,4]

Photodynamic therapy (PDT) is used for cancer treatment. This technique is based on the administration of a sensitizer devoid of mutagenic properties, followed by the exposure of the pathological area to visible light.[5] Two types of photoreaction mechanisms are invoked to explain photosensitizer action: free radical generation by electron or proton transfer, or singlet oxygen generation by energy transfer, from light activated photosensitizers. To date, the FDA has approved a photosensitizing agent, porphyrin derivative, called Photofrin® for the use in PDT.[6]

Photoactivation also affords a useful technique in life science to monitor biological processes by using so-called 'caged' compounds.[7–12] These compounds are artificial molecules whose biological activity is masked by a covalently attached photocleavable group which can be selectively removed upon light activation to release parent bioactive molecules. Recently, we applied this caged strategy for a controlled generation of intact amyloid β peptide 1–42 (Aβ1–42) for the study of Alzheimer's disease in combination with an isopeptide method to mask biological features of Aβ1–42. A synthesized phototriggered Aβ1–42 isopeptide ('click peptide') possessing a photocleavable 6-nitroveratryloxycarbonyl (Nvoc) group afforded intact Aβ1–42 with a quick and one-way conversion reaction through photoirradiation by 355 nm light and subsequent spontaneous O–N intramolecular acyl migration reaction.[13]

Taking all those three strategies (prodrug, photodynamic therapy, and caged compound chemistry) into consideration, we designed a photoresponsive targeting prodrug of paclitaxel **1**, namely phototaxel **2**, which has a coumarin derivative conjugated to an amino group

Keywords: Taxol; Tumor targeting prodrug; Photoresponsive; Coumarin; O–N intramolecular acyl migration; Visible light irradiation.
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of isotaxel (**3**, *O*-acyl isoform of paclitaxel).[14–16] This prodrug was expected to: (a) be selectively activated by visible light irradiation (430 nm) leading to cleavage of coumarin, then (b) release paclitaxel via subsequent spontaneous O–N intramolecular acyl migration reaction (Fig. 1). 7-*N*,*N*-Diethylamino-4-hydroxymethyl coumarin (**5**, DECM) was chosen as a photolabile group since DECM-caged compounds have been reported to be water-soluble, thermally stable, and rapidly photolyzed by visible light.[9,17,18]

As depicted in Scheme 1, 7-*N*,*N*-diethylamino-4-hydroxymethyl coumarin **5** was prepared from commercially available 7-*N*,*N*-diethylamino-4-methyl coumarin **4** according to the procedure reported by B. Giese and coworkers.[18] Compound **5** was activated by coupling to 4-nitrophenyl chloroformate in presence of DMAP (4-(dimethylamino)pyridine),[12] then allowed to react with 3′-*N*-debenzoylpaclitaxel, which was synthesized by a previously described method,[14,19] to afford **6**. Finally, benzoylation of the 2′-hydroxyl group with benzoic acid by the EDC-DMAP method (EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide), and following HPLC purification with ion-exchange by elution with 12 mM aq HCl gave phototaxel **2** as a HCl salt.[20]

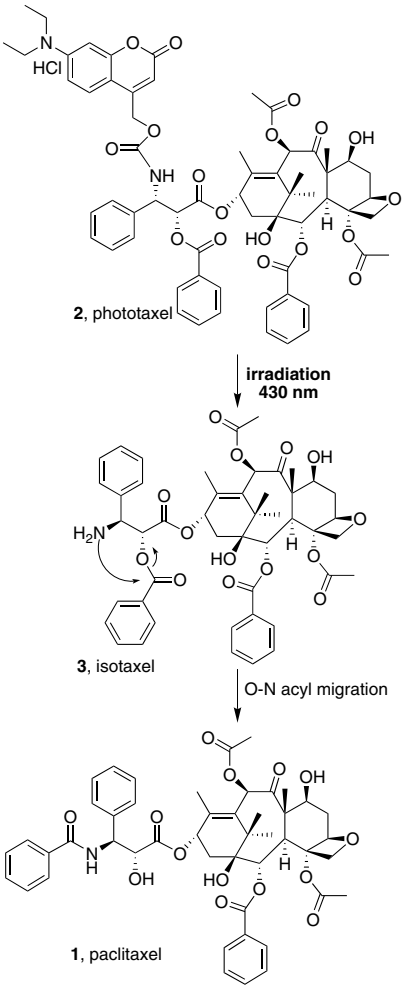
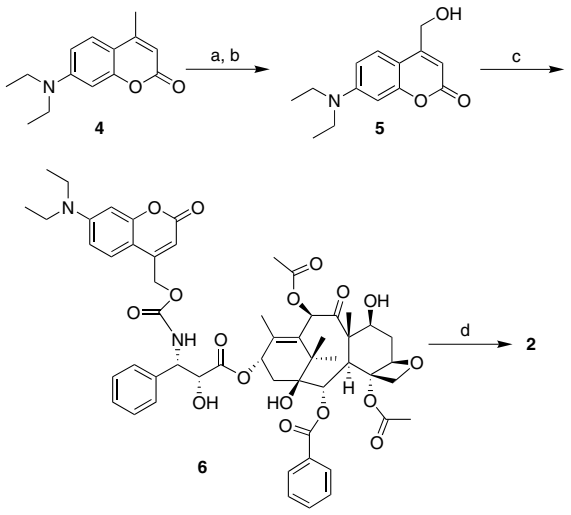


Figure 1. Releasing mechanism of paclitaxel **1** from its photoresponsive prodrug **2**.



Scheme 1. Syntheses of phototaxel **2**. Reagents and conditions: (a) SeO_2, *p*-xylene, reflux, 24 h; (b) $NaBH_4$, EtOH, rt, 4 h, 49% over two steps; (c) 4-nitrophenyl chloroformate, DMAP, CH_2Cl_2, rt, 6 h, then 3′-*N*-debenzoylpaclitaxel, DMAP, 20 h, 40%; (d) benzoic acid, EDC·HCl, DMAP, $CHCl_3$, rt, 6 h, then HCl, 70%.

Previously reported DECM-caged compounds demonstrated good water-solubility;[9,17,18] however, prodrug **2** had lower water-solubility (<0.00025 mg mL^{-1}) than parent drug **1**. Therefore, to study the kinetics of photoconversion, **2** was dissolved in a mixture of 0.1 M phosphate buffer (PB, pH 7.4) and methanol (1:1, v/v) to simulate physiological conditions[21] and to obtain concentration of prodrug enough high for the clear detection in further evaluations (20–50 μM). Prodrug **2** was photoirradiated with a diode laser (Melles Griot-85 BTL 010 laser, 430.6 nm, 10 mW) at 15 °C. The absorption spectra were taken immediately after irradiation (Fig. 2A). Photolysis of DECM-caged compound is expected to produce compound **5**,[9,18] which can be observed as a shift of long-wavelength absorption maximum to the lower wavelength (compounds **5** and **2** had intensive maxima at 385 and 393 nm, respectively). Indeed, we observed not only the shift but also a significant reduction of absorption intensity. This suggested that **2** was photolytically cleaved and the released coumarin derivative was partially decomposed. This observation was further confirmed by HPLC analysis (Fig. 2B), which was performed after irradiation followed by incubation at rt for at least 1 h to induce complete O–N intramolecular acyl migration to the parent paclitaxel (the $t_{1/2}$ value of migration of isotaxel **3** to release **1** was 15.1 min under physiological conditions).[14–16] On the HPLC charts three signals were identified by mass spectrometry analysis and confirmed with independently synthesized compounds as **5** (rt = 20.5 min), **1** (rt = 28.5 min), and **2** (rt = 36.0 min). Thus, time-dependent parent drug release from phototaxel **2** was indicated and quantitative analysis of prodrug kinetics upon photoirradiation (Fig. 3) showed that paclitaxel was released with 69% yield after 30 min; however, only 20% of **5** was recovered. Prodrug **2** was stable in the dark in phosphate-buffered saline (PBS, pH 7.4) at 4 °C for at least 1 month and as a solid state for at least 4 months at −20 °C.

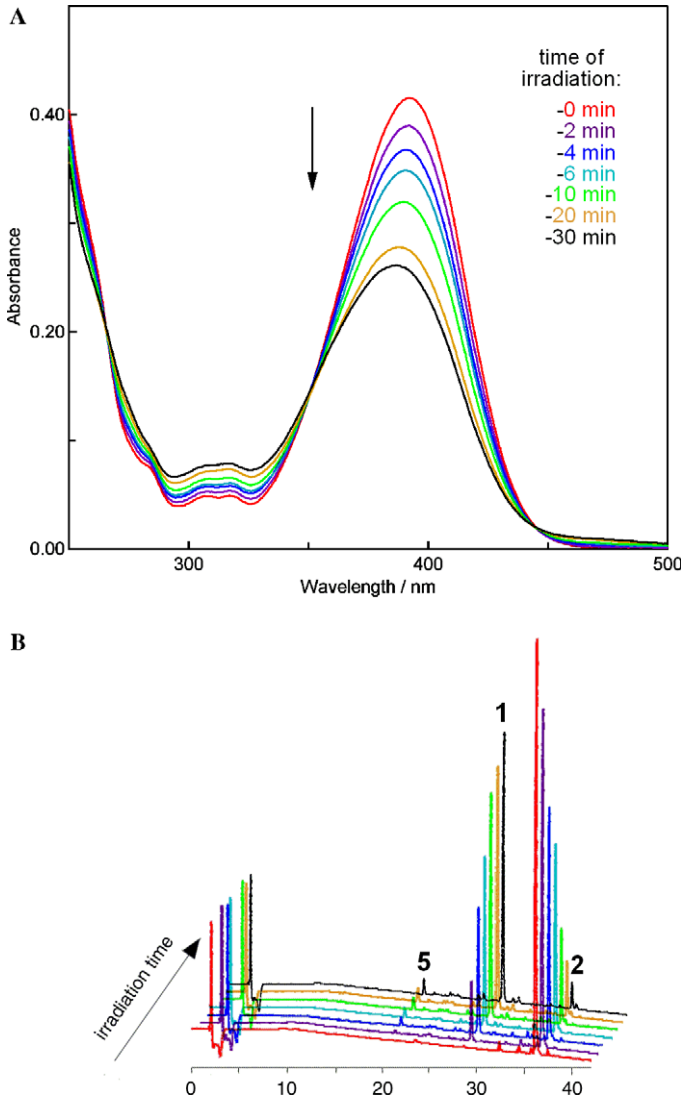


Figure 2. (A) Absorption spectra (250–500 nm) of prodrug **2** solution in PB(0.1 M)/MeOH (1:1) before (red line) and after irradiation (430.6 nm, 10 mW) for desired period of time (other lines) at 15 °C; (B) HPLC charts for prodrug **2** subjected to above irradiation conditions followed by incubation at rt to induce migration, detected at 230 nm (line colors are corresponding to irradiation time in the same manner as for absorption spectra).

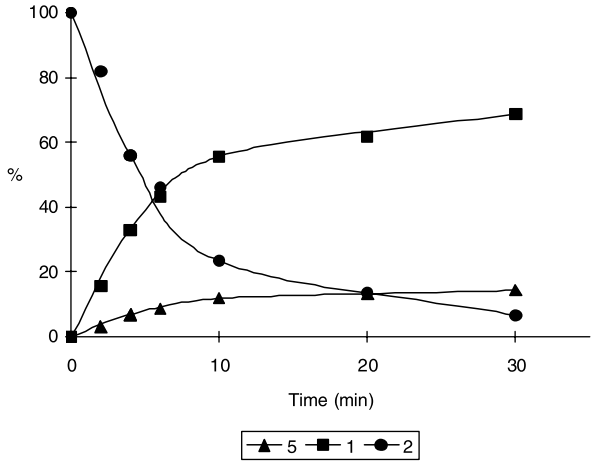


Figure 3. Time course of photolysis of prodrug **2** and release of **5** and **1** in 0.1 M PB/MeOH (1:1) at 430.6 nm. The percentage was determined by HPLC.

Extensive literature searches revealed that photolabile prodrug strategy has not yet been proposed for paclitaxel, although there are a few reports that demonstrated utilization of caged paclitaxel derivatives (activated by UV irradiation) for molecular biology related study.[22,23] Thus, herein a novel approach for developing a photoresponsive tumor targeting paclitaxel prodrug is demonstrated throughout the design and synthesis of phototaxel **2**. Prodrug **2** is not expected to be active prior to photoconversion, based on previous SAR studies on paclitaxel derivatives.[24,25] Namely, it is known that both extensive modifications of the *N*-acyl moiety and masking of the 2′-OH group restrain paclitaxel activity.[25] Upon visible light irradiation (430.6 nm, 10 mW) this prodrug released isotaxel **3** (half-life of prodrug was 4.8 min, Fig. 3), and subsequent spontaneous O–N intramolecular acyl migration ($t_{1/2}$ = 15.1 min)[14–16] formed intact paclitaxel **1**. This delay of parent drug release after irradiation (related to migration of benzoyl

group) is supposed to be short enough to avoid the intermediate (**3**) diffusion from the photoirradiated site before the parent drug is released. Moreover, we recently demonstrated faster O–N intramolecular acyl migration in other highly potent taxoids.[14,15,19] For example, prodrug of canadensol (3′-*N*-isopropylcarbonyl-3′-*N*-debenzoylpaclitaxel) had a $t_{1/2}$ value of 4.3 min under physiological conditions.[15,19] 2′-*O*-Benzyloxycarbonyl-3′*N*-debenzoyl-paclitaxel, prodrug design based on the O–N intramolecular alkoxycarbonyl migration reaction,[26] exhibited even instantaneous conversion to a parent carbamate-type taxoid ($t_{1/2} < 1$ min).[14] Phototaxoids derived from these types of taxoids would be more effective without risking diffusion from the irradiation site.

The observed recovery yield (69%) of paclitaxel **1** in the photo-triggered conversion of **2** in HPLC analysis is in agreement with previous reports on caged compounds (typical released yield was 40–70%).[7,27,28] This relatively moderate recovery might be related to partial decomposition of prodrug **2** or intermediate **3** due to their photoinstability, as we observed low recovery of coumarin derivative **5** from prodrug **2** contrary to a previous report[18] (Figs. 2 and 3). However, no major byproduct formation was detected by HPLC, and paclitaxel **1** was almost stable under the photo-irradiation conditions used for conversion of prodrug **2**, that is, only a small amount of paclitaxel decomposition (about 2%) was observed by photo-irradiation for 0.5 h (data not shown). Another reason could be non-specific absorption of compounds on the surface of experimental tubes.

DECM **5** has been chosen as a photolabile group, as much less expensive and simple-to-use light sources are available for experiments in the visible wavelength region. In spite of intensive maxima of DECM-caged compounds are in UV-range (around 390 nm), these compounds have been activated by visible lights (even by irradiation at 436 nm).[9,18] Moreover, during our initial experiments on prodrug **2** irradiated with UV pulses (355 nm, 10 Hz, 5–20 mJ), extensive decomposition of **2** was observed (data not shown). In contrast, irradiation at 430.6 nm showed to be effective for triggering parent drug release without any major decomposition of prodrug or parent drug.

In conclusion, we designed and synthesized a new photoresponsive paclitaxel prodrug based on an idea that combined both photodynamic cancer therapy and caged chemistry. The prodrug, phototaxel **2**, released parent drug, paclitaxel, with a reasonable conversion time by visible light irradiation suggesting that this strategy is practically applicable for wide range of anticancer agents to develop new photoresponsive prodrugs. This would expand the current photodynamic therapy which is dependent on photosensitizers (porphyrin derivatives) that generate free radicals or singlet oxygen as cytotoxic spices.

Acknowledgments

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20. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.03 (d, J = 4.5 Hz, 2H), 7.97 (d, J = 6.6 Hz, 2H), 7.61 (t, J = 6.8 Hz, 1H), 7.52–7.21 (m, 10H), 7.02 (br s, 1H), 6.51–6.38 (m, 3H), 6.30 (s, 1H), 6.00 (br s, 2H), 5.67 (d, J = 7.3 Hz, 1H), 5.66–5.61 (m, 2H), 4.27, 4.07 (2d, J = 14.7 Hz, 2H), 4.97 (d, J = 8.1 Hz, 1H), 4.46 (dd, J = 6.5, 10.9 Hz, 1H), 4.24, 4.21 (2d, J = 8.7 Hz, 2H), 3.83 (d, J = 7.0 Hz, 1H), 3.39 (br s, 4H), 2.62–2.51 (m, 1H), 2.45 (s, 3H), 2.36–2.30 (m, 1H), 2.24 (s, 3H), 2.19–2.07 (m, 1H), 2.00 (s, 3H), 1.92–1.85 (m, 1H),

1.69 (s, 3H), 1.30 (s, 3H), 1.20 (br s, 6H), 1.15 (s, 3H). HRMS (FAB+): calcd for $C_{62}H_{66}N_2O_{14}$ [M^++Na]: 1149.4208, found: 1149.4202. Purity was higher than 95% (HPLC analysis at 230 nm).

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