DOI: 10.1002/ejoc.200700972

A Model for Light-Triggered Porphyrin Anticancer Prodrugs Based on an *o*-Nitrobenzyl Photolabile Group

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Keywords: Anticancer / Prodrugs / Porphyrinoids / Light-triggered compounds / Cytotoxicity

A model for light-triggered porphyrin anticancer prodrug **1** was designed and synthesized. Upon photolysis, prodrug **1** can efficiently liberate the anticancer drug tegafur. The MTT assay demonstrates that prodrug **1** is significantly less toxic than its parent drug tegafur, and **1** can release tegafur upon photoactivation in vitro. The light-triggered porphyrin anticancer prodrug technique developed herein may find useful applications in chemotherapy to minimize the side effects of anticancer drugs because of the tumor affinity property of porphyrin and the light-controllable anticancer drug dosing.

Introduction

Porphyrins are a class of photosensitizers used in photodynamic therapy. Upon irradiation by red light (around 650 nm), porphyrins may be initially excited and further result in the formation of cytotoxic species such as singlet oxygen. One important feature of porphyrins as photodynamic therapy agents is that they tend to accumulate in neoplastic tissue to higher concentrations than in surrounding normal tissue.[1–6] The tumor-affinity property of porphyrins has been exploited in anticancer drug design by integrating porphyrins with drugs to prepare porphyrin–drug conjugates: Brunner et al. created a series of porphyrin–platinum conjugates by combining porphyrin with cytotoxic platinum complexes;[7,8] Zhou reported some porphyrin–DNA-alkylation-agent conjugates;[9] We also developed a series of porphyrin conjugates as anticancer agents by linking porphyrin with nitrogen heterocyclic species.[10,11] However, all the porphyrin–drug conjugates reported so far lack a drug releasing mechanism, so they could not release drugs from the conjugates. Obviously, a true prodrug should be able to liberate the biologically active drug from the prodrug. There is a need to develop porphyrin anticancer prodrugs that are able to release the anticancer drug.

As photolysis of light-triggered ("caged") compounds can be temporally and spatially controlled,[12,13] light-triggered compounds have been employed as a significant tool in biological and medicinal studies.[12–22] For example, Diamond reported the photocleavage of *o*-nitrobenzyl ether derivatives for biomedical release applications,[15] Reinhard developed a series of *o*-nitrobenzyl-based photosensitive phosphoramide mustards,[16] Gong exploited a model of a photoactivated prodrug,[17] and Kehayova synthesized a caged hydrophobic inhibitor of carbonic anhydrase II.[18] Very recently, the group of McCoy provided an interesting example of drug dosing with precise control by photolysis of light-triggered compounds with the use of UV light.[19] The major concern of the application of a photochemical system in medicine is the tissue-damaging effect of UV light. However, long-wavelength UV light generally imparts minimal damage to tissues.[19,22] In addition, electromagnetic radiation can be transferred by a fiber optic to further minimize potential damage in medicinal applications.[19,17]

To the best of our knowledge, no examples of porphyrin anticancer prodrugs that are able to release anticancer drugs have ever been disclosed in the literature. In addition, although a light-triggered strategy has been applied in the design of the prodrug,[16–19] there is no report of light-triggered anticancer prodrugs with a porphyrin. To continue our efforts in the development of porphyrin anticancer prodrugs,[10,11] in this communication, we present the first paradigm of an anticancer prodrug: it contains a porphyrin,

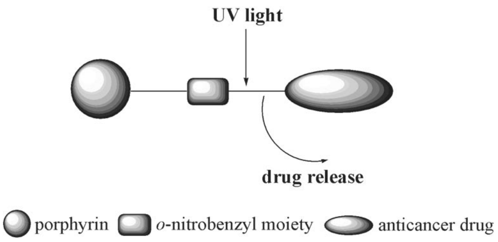

Figure 1. Schematic representation of the light-triggered porphyrin anticancer prodrug.

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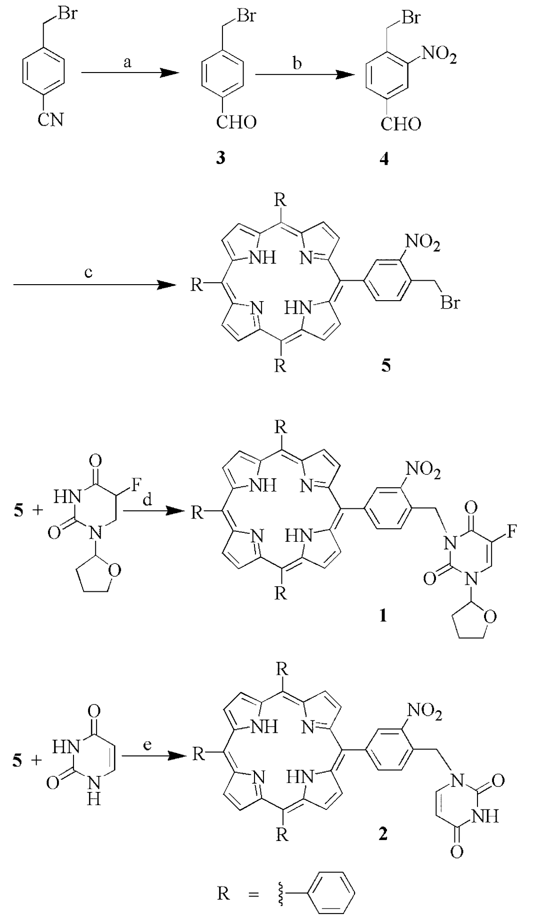
but it is also able to release anticancer drugs upon photolysis by long-wavelength UV light. The porphyrin anticancer prodrug developed herein is composed of three parts: a porphyrin, a photocleavable *o*-nitrobenzyl moiety as a light-triggered group, and a parent anticancer drug (Figure 1). As a proof of concept, compound **1** (Scheme 1) was chosen as the model for the light-triggered porphyrin anticancer prodrugs. We describe herein the synthesis, photochemistry, and cytotoxic evaluation of porphyrin anticancer prodrug **1**.



Scheme 1. Synthetic route to prodrug **1** and control **2**. Reagents and conditions: (a) DIBAL-H, toluene, 0 °C, 2.5 h; (b) KNO_3, H_2SO_4, 0 °C, 2.5 h; (c) benzaldehyde, pyrrole, $BF_3{\cdot}Et_2O$, $CHCl_3$, room temp., 5 h, *p*-chloranil, 60 °C, 2 h; (d) K_2CO_3, anhydrous DMF, 70 °C, 10 h; (e) K_2CO_3, anhydrous DMF, 50 °C, 8 h.

Results and Discussion

A versatile synthetic route to light-triggered prodrug **1** was developed (Scheme 1). We employed 4-bromomethylbenzonitrile as the starting material to prepare key intermediate **4**. 4-Bromomethylbenzonitrile was chemoselectively reduced by diisobutylaluminiumhydride (DIBAL-H) in toluene under a nitrogen atmosphere at 0 °C to give 4-bromomethylbenzaldehyde (**3**).[23–25] Toluene instead of chlorobenzene was chosen as the solvent for easier workup.[24] Nitration of **3** with a mixture of KNO_3 and H_2SO_4 smoothly produced key intermediate **4** in 78.6% yield. Porphyrin derivative **5** was prepared in 10.5% yield by treating aldehyde **4** (1 equiv.) with benzaldehyde (4 equiv.) and pyrrole (5 equiv.) under standard Lindsey conditions.[26] A statistical mixture of porphyrins was produced by this procedure, and desired compound **5** was separated from the other porphyrin products by flash chromatography over silica gel. With compound **5** in hand, the stage was set to prepare target compound **1**. Alkylation of intermediate **5** with anticancer drug tegafur (widely used in cancer treatment), in the presence of potassium carbonate at 70 °C afforded **1** in 92.6% yield. Uracil, a noncytotoxic compound, was used to replace anticancer drug tegafur in control **2**. Similar nucleophilic substitution reaction conditions were employed to prepare control **2** by treating intermediate **5** with uracil. For uracil, the nucleophilic substitution reaction preferably occurred at the N-1 position.[27,28] After initial purification by silica-gel chromatography, target compound **1** and control **2** were further purified by preparative HPLC to finally afford a single peak in the HPLC chromatogram. Compound **1** and control **2** were both characterized by NMR, mass, and UV spectroscopy and elemental analysis.

Preliminary assessment of the light-triggered conversion of anticancer prodrug **1** to anticancer drug tegafur was performed. The solution of prodrug **1** was irradiated with long-wavelength UV light (350 nm). Aliquots were subsequently analyzed by using reverse-phase HPLC. The conversion of prodrug **1** into tegafur was followed as a function of time, as shown in Figure 2. Prodrug **1** displayed rapid photoliberation to tegafur. For example, after irradiation for 12 min, approximately 50% of prodrug **1** was photolyzed to release tegafur. The photolysis byproduct was identified as 5-(3-nitroso-4-formylphenyl)-10,15,20-triphenylporphyrin {MS (ESI): $m/z = 672.3\ [M + H]^+$}, which is consistent with the photolysis mechanism of the *o*-nitrobenzyl group.[29] The photochemical quantum yield ($\phi = 0.032$) of the photolysis was determined by ferrioxalate actinometry.[30] Although the *o*-nitrobenzyl moiety is linked to the N-1 position of uracil in control **2**, according to the photolysis mechanism of *o*-nitrobenzyl compounds,[29] control **2** should release uracil (the same way as prodrug **1** releases anticancer drug

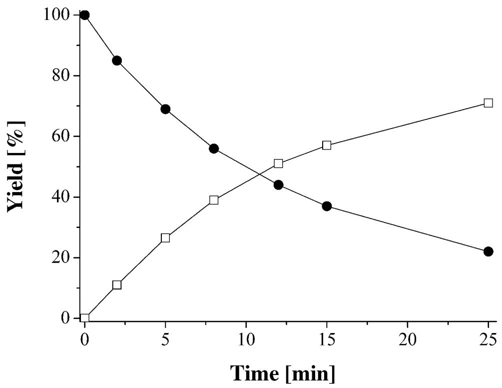


Figure 2. Photolysis conversion of prodrug **1** (●) and formation of tegafur (□).

tegafur) upon photolysis. Indeed, control **2** was also photolyzed to release uracil efficiently.

As the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay has been widely used to evaluate the cytotoxicity of drugs,[10,11,31,32] we decided to also employ the MTT assay to examine the cytotoxicity of anticancer prodrug **1** in the absence or presence of long-wavelength UV light (350 nm). MCF-7 mammary cancer cells were incubated with anticancer prodrug **1** or the anticancer drug tegafur. As expected, tegafur induced up to 91 % cell death (Figure 3). By contrast, prodrug **1** induced only 7 % cell death; this indicated that prodrug **1** has significantly less cell toxicity than tegafur. However, when MCF-7 mammary cancer cells were incubated with prodrug **1** preirradiated with UV light for 25 min, approximately 67 % cell death was observed. Similarly, irradiation of cells treated with prodrug **1** for 25 min induced 69 % cell death. This is consistent with the fact that irradiation for 25 min converted roughly 70 % of prodrug **1** into tegafur. A control experiment of UV light indicated that irradiation for 25 min by long-wavelength UV light alone (in the absence of prodrug **1** or tegafur) did not induce marked effects on cell viability. As porphyrins may produce cytotoxic singlet oxygen when irradiated by red light (around 650 nm) in photodynamic therapy, it is necessary to examine whether the photolysis-induced cytotoxicity (photolysis at 350 nm) of prodrug **1** is due to the production of singlet oxygen or the release of the anticancer drug. Towards this end, control **2**, which contains noncytotoxic uracil instead of an anticancer drug, was incubated with cells and irradiated at 350 nm for 25 min, but only approximately 6 % cell death was observed. Therefore, the cytotoxicity of control **2** upon photolysis at 350 nm UV light is similar to that with 350 nm UV light alone. These results unambiguously demonstrated that the photolysis-induced cytotoxicity (photolysis at 350 nm) observed for prodrug **1** was due to the release of the anticancer drug, and the photolysis byproduct, aromatic nitroso, did not appear to induce apparent deleterious effects on the viability of the cell (probably due to the high intracellular concentration of reduced glutathione, which may neutralize the byproduct).[13,33,34]

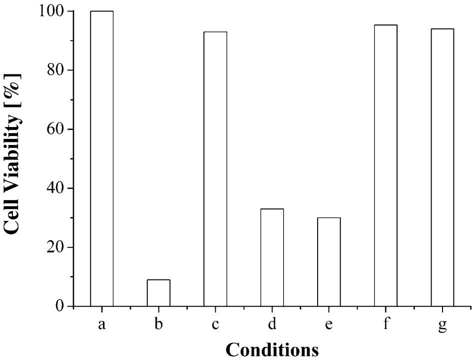


Figure 3. MCF-7 mammary cancer cell viability under different conditions. Experimental conditions: (a) cells; (b) 50 μM tegafur + cells; (c) 50 μM prodrug **1** + cells; (d) 50 μM prodrug **1** +(pre)*hν*, 25 min + cells; (e) 50 μM prodrug **1** + cells + *hν*, 25 min; (f) cells + *hν*, 25 min; (g) 50 μM control **2** + cells + *hν*, 25 min.

Conclusions

A versatile synthetic route was developed to prepare anticancer prodrug **1**, which upon photolysis can be efficiently converted into the anticancer drug tegafur. The MTT assay demonstrated that prodrug **1** is significantly less toxic than its parent anticancer drug tegafur, and prodrug **1** can release the cytotoxic anticancer drug tegafur upon photoactivation in vitro. Furthermore, the control experiments also demonstrated that the photolysis-induced cytotoxicity (photolysis at 350 nm) observed for prodrug **1** is due to the release of the anticancer drug. As we are aware, prodrug **1** developed herein represents the first paradigm of a porphyrin anticancer prodrug that is able to release anticancer drugs and the first example of light-triggered anticancer prodrug with a porphyrin. We envision that, combined with the up-to-date medical fiber optic technique, the light-triggered porphyrin anticancer prodrug may find useful applications in chemotherapy to minimize side effects of anticancer drugs because of the tumor-affinity property of porphyrin[1–6] and the light-controllable anticancer drug dosing.[19] In addition, the light-triggered porphyrin anticancer prodrug technique created herein may potentially be employed as binary cancer treatment in both chemotherapy and photodynamic therapy to potentiate the efficacy of anticancer drugs. Because two-photon excitation has better tissue penetration than UV excitation, and because it could also minimize the phototoxicity to cells and tissues,[35–38] for practical use of this light-triggered porphyrin anticancer prodrug technique, the UV photolabile *o*-nitrobenzyl moiety will be replaced with a two-photon photolabile species such as a 7-hydroxycoumarin moiety,[35] and the results will be reported in due course.

Supporting Information (see footnote on the first page of this article): Procedures for the preparation, photolysis, and in vitro cytotoxicity tests for compound **1**.

Acknowledgments

Funding was partially provided by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry (2007-24), the Key Project of Chinese Ministry of Education, and the Hunan University research funds.

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Received: October 16, 2007