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Synthesis and preliminary cytotoxicity study of a cephalosporin-CC-1065 analogue prodrug

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Abstract

Background: Antibody-directed enzyme prodrug therapy (ADEPT) is a promising new approach to deliver anticancer drugs selectively to tumor cells. In this approach, an enzyme is conjugated to a tumor-specific antibody. The antibody selectively localizes the enzyme to the tumor cell surface. Subsequent administration of a prodrug substrate of the enzyme leads to the enzyme-catalyzed release of the free drug at the tumor site. The free drug will destroy the tumor cells selectively, thus, reducing side effects.

Results: A CC-1065 analogue was conjugated to a cephalosporin affording prodrug **2**. The prodrug and its corresponding free drug, **1**, have IC_{50} values of 0.9 and 0.09 nM, respectively, against U937 leukemia cells in vitro.

Conclusions: For the first time, a prodrug comprised of a cephalosporin and a CC-1065 analogue has been synthesized. The preliminary in vitro studies show that the prodrug was 10-fold less toxic than the free drug. Prodrug **2** has the potential to be useful in cancer treatment using the ADEPT approach.

Background

Antibody-directed enzyme prodrug therapy (ADEPT) [1–5] is one of the promising new approaches that selectively target tumor cells, thus reducing toxic side effects to patients. In this approach, an enzyme is conjugated to a tumor-specific antibody. The antibody selectively localizes the enzyme to the tumor cell surface. Subsequent administration of a prodrug substrate of the enzyme leads to the enzyme-catalyzed release of the free drug at the tumor site. This strategy addresses the stoichiometry, controlled drug release and poor antibody penetration problems associated with the use of monoclonal antibody-drug conjugates [6–8]. In addition, because the process of drug release is enzymatic, a single enzyme can generate a large amount of free drug. Consequently, a small amount of antibody can be used to reduce immunogenicity.

It is important that the free drug in the ADEPT approach be highly toxic. Using highly toxic agents can reduce the amount of the monoclonal antibody required, thereby reducing side effects. CC-1065 (Figure 1) is among the most potent antitumor agents discovered [9–13]. It binds to double-stranded B-DNA within the minor

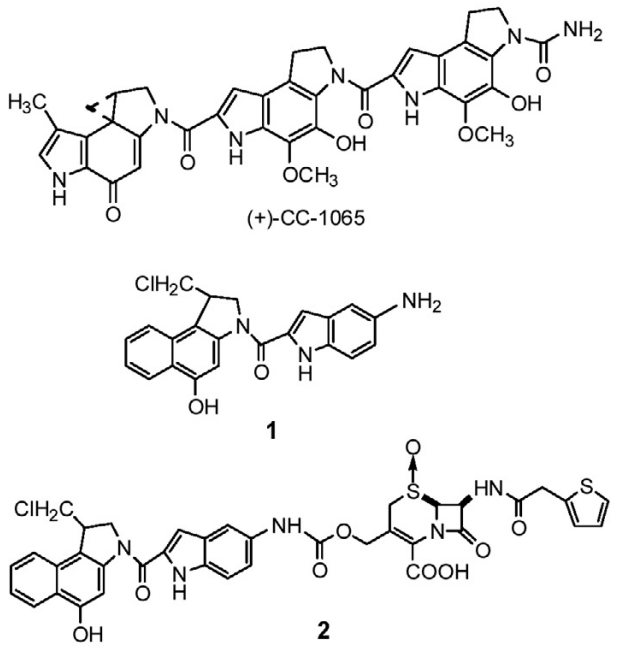


Figure 1
Structures of CC-1065 and related Compounds

groove with a sequence preference for 5'-d(A/GNTTA)-3' and 5'-d(AAAAA)-3', and alkylates the N3 position of the 3'-adenine with its left-hand CPI segment [14,15]. CC-1065 also inhibits gene transcription by interfering with binding of the TATA box-binding protein to its target DNA [16]. Despite its high potency and broad spectrum of antitumor activity, CC-1065 cannot be used in humans because it causes delayed death in experimental animals [17]. To pursue compounds possessing the potent antitumor activity but devoid of the toxic side effects of the parent compound, many CC-1065 analogues have been synthesized [18–26].

Beta-lactamases have been widely investigated for their role in the metabolism of antibiotics including cephalosporins and penicillins. Because of the high catalytic efficiency and broad substrate specificity, β-lactamases have been extensively used in the ADEPT approach to activate prodrugs of vinca alkaloids [27], nitrogen mustard [28–32], doxorubicin [33–36] and others [37]. To take advantage of the potent antitumor activity of the CC-1065 class of compounds and the ADEPT approach, we have synthesized β-lactam prodrugs. Herein, we report synthesis and preliminary cytotoxic effects of a prodrug comprised of a cephalosporin and a CC-1065 analogue (Figure 1).

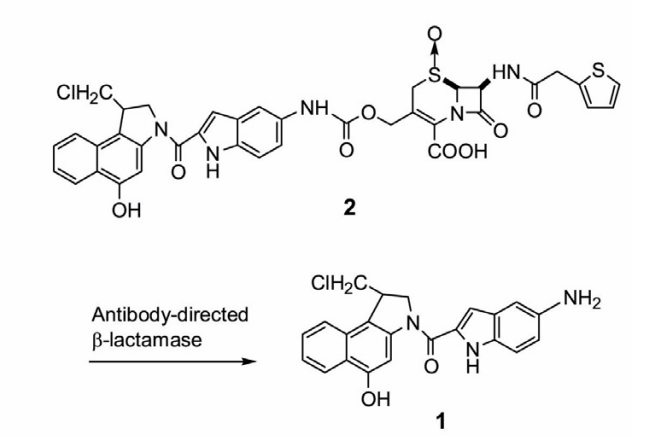


Figure 2
Activation of prodrug to free drug

Prodrug **2** is expected to be less toxic than its corresponding free drug **1**. However, it is expected that the prodrug will be converted to the potent free drug by β-lactamases localized on the tumor cell surface by an antibody (Figure 2). This selective activation of prodrug **2** at the tumor site will lead to enhanced antitumor therapeutic efficacy.

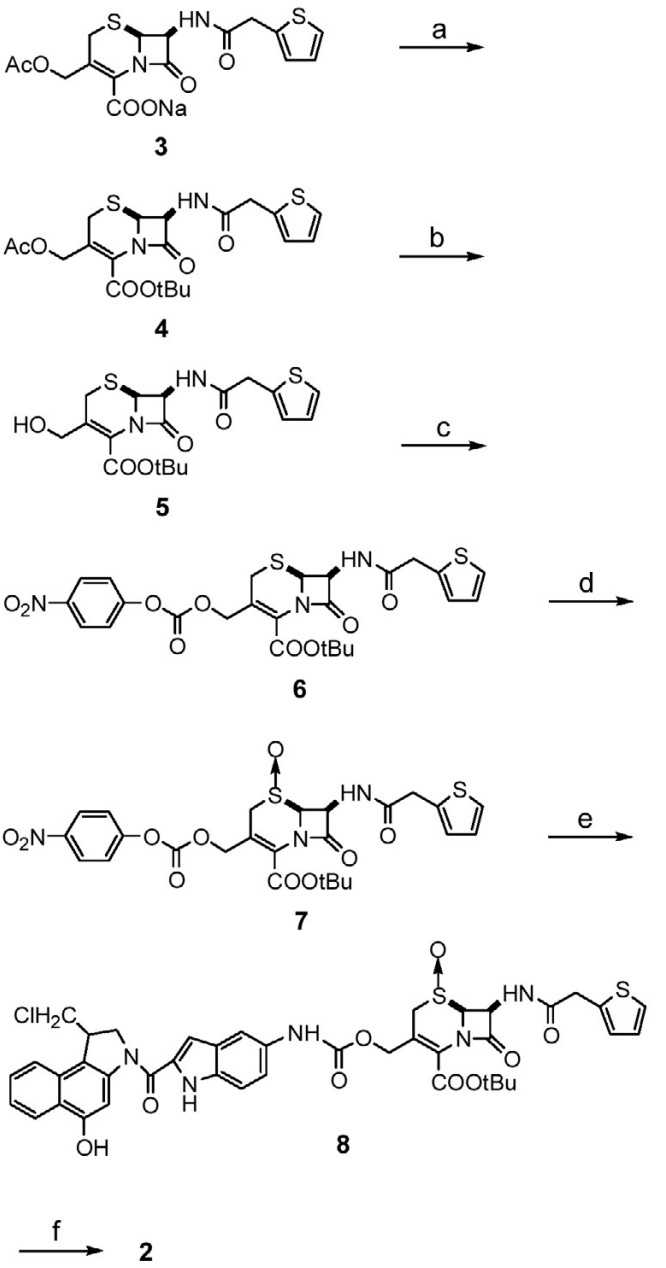
Results and discussion

Prodrug **2** was synthesized as shown in Figure 3. The key intermediate, **7**, was made using methods developed by Jungheim et al. [33], and Rodrigues, et al. [35,36] with modifications. The spectra data including NMR and MS of compounds **4**–**7** were identical to those as reported. Compound **1** was treated with **7** in DMF to afford the protected prodrug **8**. Removal of the t-butyl protection group from **8** generated the targeted prodrug **2** with good yield.

The cytotoxicity of prodrug **2** and its corresponding free drug **1** was tested against U937 leukemia cells, and the results are presented in Table 1. When the drugs were incubated with U937 cells for a period of 48-h, prodrug **2** (IC_{50}: 0.9 nM) is 10-fold less toxic than its corresponding free drug **1** (IC_{50}: 0.09 nM). As observed for other compounds of the CC-1065 class [25,37,38], both prodrug **2** and the free drug **1** caused DNA fragmentation, and the cells died by apoptosis (data not shown).

Conclusion

This is the first report demonstrating synthesis of a prodrug comprised of a cephalosporin and a CC-1065 analogue. The preliminary in vitro studies show the prodrug to be less toxic than the free drug. Due to the slow non-enzymatic degradation of the cephalosporins in solution [39], the ratio of toxicity of cephalosporin-containing



(a). Anhydrous HCl/dioxane, CH_2Cl_2;
(b) *tert*-butyl trichloroacetimidate;
(c) K_2CO_3, MeOH;
(d) *p*-nitrophenylchloroformate, 2, 6-lutidine, DMAP;
(e) 55% *m*-CPBA, CH_2Cl_2;
(f) **1**, DMF;
(g) TFA, DMF, CH_2Cl_2.

Figure 3
Synthesis of prodrug 2

Table 1 : Cytotoxicity of compounds 1 and 2 against U937 leukemia cells in vitro[a]

Compd.	IC_{50} (nM)[b]
1	0.09
2	0.9

[a]Cells were incubated with drugs for 48 h and the experiments were performed according to our previously published method;[27b]IC_{50} values are defined as the minimal drug concentration necessary to inhibit incorporation of [^{3}H]thymidine by 50%, and are the averages of three experiments.

prodrugs to their corresponding free drugs is generally not very high. However, some of the prodrugs are very effective against tumors in mouse models. For example, a cephalosporin-doxorubicin prodrug was 9-fold less toxic than free doxrubicin against tumor cells in vitro, but caused tumor regression when tested in tumor xenograft models [40]. A cephalosporin-vinca alkaloid prodrug was 5-fold less toxic than the free drug against tumor cells in vitro, but was highly effective in tumor xenograft models in vivo [41]. When taxol was conjugated to a cephalosporin, the resulting prodrug was approximately 10-fold less toxic than free taxol against tumor cells in vitro [36]. Thus, prodrug **2** has the potential to be useful in cancer treatment using the ADEPT approach. We will report more biological data in due course.

Materials and methods

Cephalothin sodium, **3**, (2.5 g, 5.98 mmol) was suspended in dichloromethane (150 mL). Anhydrous hydrogen chloride (4 N in dioxane, 2 mL, 8 mmol) was added, and the reaction mixture was stirred for 30 min at room temperature. *tert*-Butyl trichloroacetimidate (3.2 mL, 17.84 mmol) was added, and the reaction mixture was stirred overnight at room temperature. The reaction mixture was washed consecutively with water (150 mL), saturated sodium hydrogen carbonate solution (150 mL) and water (150 mL). The organic solution was dried using sodium sulfate. The solvent was removed, and the product was purified by flash column chromatography eluting with a solvent consisting of dichloromethane, ethyl acetate and hexane (1/1/3, v/v) affording 1.2 g of **4** (44% yield).

Compound **4** (1 g, 2.21 mmol) was dissolved in methanol (70 mL), and solid potassium carbonate (120 mg) was added. The mixture was stirred for 2 h at room temperature, and acetic acid (200 μL) was added to quench the reaction. The solvent was removed, and the product was purified by flash column chromatography eluting with 8% acetone in dichloromethane to afford 220 mg of **5** (24% yield).

Compound **5** (280 mg, 0.68 mmol) was dissolved in anhydrous THF (40 mL), and dimethylaminopyridine (1 mg), p-nitrophenylchloroformate (0.2 g, 1 mmol) and 2, 6-lutidine (120 μL), 1 mmol) were added sequentially. The reaction mixture was stirred overnight at room temperature. The solvent was removed, and the product was purified by flash column chromatography eluting with 5% ethyl acetate in dichloromethane to afford 271 mg of **6** (69% yield).

To a solution of **6** (50 mg, 87 μmol) in dichloromethane (2 mL) cooled to 0°C was added *m*-chloroperoxybenzoic acid (CPBA, 26 mg, 93 μmol) in 0.5 mL of dichloromethane. The reaction mixture was stirred for 15 min at 0°C, and was then washed with 5% potassium hydrogen carbonate solution followed by brine. The solvent was removed, and the product was purified by flash column chromatography eluting with 8% ethyl acetate in dichloromethane to afford 34 mg of **7** (66% yield).

Compound **7** (15 mg, 25 μmol) was added to a solution of **1** (9 mg, 23 μmol) in DMF (0.3 mL), which was synthesized as we reported previously [20], and the reaction mixture was stirred overnight at room temperature. The product was purified by thin layer chromatography eluting with ethyl acetate and hexane (3/1, v/v) to afford 12 mg of **8** (62% yield). ^{1}H NMR (DMF-d_7, ppm): 10.70 (s, 1 H), 9.15 (s, 1 H), 8.63 (s, 1 H), 8.25–7.85 (m, 4 H), 7.60–7.19 (m, 7 H), 7.05–6.95 (m, 2 H), 6.05–6.01 (m, 1 H), 5.39–5.30 (d, 1 H), 5.12–4.79 (m, 5 H), 4.35–4.27 (m, 1 H), 4.19–3.75 (m, 6 H), 1.58 (s, 9 H). FAB MS *m/e* 866.0 $(M + Na)^+$.

To a solution of **8** (5 mg, 5.9 μmol) in DMF (0.2 mL) and dichloromethane (1 mL) was added trifluroacetic acid (1 mL), and the solution was stirred for 2 h at room temperature. The solvent was removed, and ethyl ether was added. The solid was filtered, and washed with ether to afford prodrug **2** (3.7 mg, 79% yield). ^{1}H NMR (DMF-d_7, ppm): 11.56 (s, 1 H), 10.50 (s, 1 H), 9.65 (s, 1 H), 8.25–7.85 (m, 4 H), 7.60–7.24 (m, 7 H), 7.10–6.96 (m, 2 H), 6.10–6.01 (m, 1 H), 5.42–5.38 (d, 1 H), 5.10–4.60 (m, 5 H), 4.35–4.25 (m, 1 H), 4.20–3.75 (m, 6 H). FAB MS *m/e* 787.1.

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