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 IC50 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.
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 1
Structures of CC-1065 and related Compounds

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
Figure 3
Synthesis of prodrug 2

(a). Anhydrous HCl/dioxane, CH₂Cl₂;
(b) tert-butyl trichloroacetimidate;
(c) K₂CO₃, MeOH;
(d) p-nitrophenylchloroformate, 2, 6-lutidine, DMAP;
(e) 55% m-CPBA, CH₂Cl₂;
(f) 1, DMF;
(g) TFA, DMF, CH₂Cl₂.
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 doxorubicin 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 then was 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). 1H NMR (DMF-d7, 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/z 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 trifluoroacetic 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). 1H NMR (DMF-d7, 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/z 871.1.

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