Synthesis and cytotoxicity of a biotinylated CC-1065 analogue
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Abstract

Background: The use of pretargeting technology for cancer imaging and treatment has made significant progress in the last few years. This approach takes advantage of the fact that biotin binds strongly to proteins avidin and streptavidin. Thus, a non-toxic tumor cell specific antibody is conjugated with avidin/streptavidin, and is administered to patients. After the antibody binds to tumor cells (usually 24–48 h), a clearing agent is given to remove the residual circulating antibodies in blood. Lastly, a toxic biotin-radioisotope conjugate is administered. Due to the small size of the biotin-radioisotope molecule and tight binding between biotin and avidin/streptavidin, the biotin-radioisotope rapidly binds to tumor cells with high specificity. CC-1065 (1) is one of a few classes of extremely potent antitumor agents, and a biotinylated CBI-bearing CC-1065 analogue is a promising candidate to be used in the pretargeting technology to treat cancer.

Results: A biotinylated CBI-bearing CC-1065 analogue, 6, was synthesized. The IC_{50} of 6 was 0.7 nM against U937 cells. Compound 6 caused apoptosis of U937 cells.

Conclusions: For the first time, a biotinylated CBI-bearing CC-1065 analogue, 6, was synthesized. The biotinylated 6 can serve as a model compound to explore the usefulness of non-radioactive small molecule anticancer drugs in the pretargeting strategy for cancer imaging and therapy.

Background

Anticancer drugs generally act on metabolically active or rapidly proliferating cells, and cannot distinguish between cancer and normal cells; thus, toxicities to normal cells limit the dose of drugs that can be given to patients. Therefore, much research has focused on development of more specific therapeutic strategies to reduce toxicity to normal cells. One of these strategies is monoclonal antibody-directed pretargeting technology. The use of pretargeting technology for cancer imaging and treatment has made significant progress in the last few years [1–6]. This approach takes advantage of the fact that biotin binds strongly to proteins avidin and streptavidin [7,8]. Thus, a non-toxic tumor cell specific antibody is conjugated with avidin/streptavidin, and is administered to patients. After the antibody binds to tumor cells (usually 24–48 h), a clearing agent is given to remove the residual circulating antibodies in blood. Lastly, a toxic biotin-radioisotope conjugate is administered. Due to the small size of the biotin-radioisotope molecule and tight binding between biotin and avidin/streptavidin, the biotin-radioisotope rapidly binds to tumor cells with high specificity. This ap-
approach decouples the lengthy antibody to target process and the administration of a toxic moiety, but takes full advantage of both the high target specificity of the antibody and the favorable pharmacokinetics of a small toxic molecule. While progress using radioisotopes in this approach has been achieved, few reports on using a potent non-radioactive small molecule anticancer drug for this purpose have been seen. A potent non-radioactive small molecule drug for this approach is potentially better than a radioisotope because the latter is much more difficult to handle than a non-radioactive chemical agent.

CC-1065 (1) is one of a few classes of extremely potent antitumor agents [9–12]. It binds to double-stranded B-DNA within the minor groove with the 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 [13–15]. 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 retaining the potent antitumor activity but devoid of the toxic side effects of the parent compound, many CC-1065 analogues have been synthesized and tested [18–26].

Because of the high potency, broad spectrum of antitumor activity, and novel mechanism of action, the CC-1065 class of compounds has the potential to be useful in the pretargeting approach. Herein, we report synthesis and preliminary cytotoxicity study of a CBI-biotin CC-1065 analogue designed as a model compound to explore its use in the pretargeting approach for cancer imaging and therapy.

### Results and discussion

Target compound 6 was synthesized using two methods. The synthesis using Method A is illustrated in Scheme 1. Alternatively, 6 was also synthesized using Method B (Scheme 2).

Using Method A, a radioisotope-labeled N-hydroxysuccinimidobiotin can be conveniently added at the last step to synthesize radioisotope-labeled materials for metabolic and other biological studies. Because of the lengthy process and low overall yield in the synthesis of starting material 2, Method B is more economical for large-scale synthesis.

The cytotoxicity of 5, 6, and doxorubicin, used as a positive control, were tested against U937 leukemia cells, and the results were presented in Table 1. The biotinylated 6 (IC50: 0.7 nM) is approximately 7-fold less toxic than its precursor 5 (IC50: 0.09 nM). However, 6 is still much more potent than doxorubicin (IC50: 100 nM). As observed for other CC-1065 class of compounds [26,28,29], 6 caused DNA fragmentation and the cells died by apoptosis (data not shown).

### Conclusions

The preliminary in vitro studies suggest that a biotin moiety can be incorporated into a CBI-bearing CC-1065 analogue to produce a potent biotinylated agent. When a biotin-nuclide conjugate is used, the conjugate does not need to be internalized to destroy the tumor cells. However, for a biotinylated CC-1065 analogue to work in this pretargeting strategy, the biotinylated drug must first bind to the avidin/streptavidin-antibody conjugate on the tumor cell surface. The drug must then gain entry to the cell to act by binding to DNA. Whether compound 6 can be internalized, after binding to the avidin/streptavidin-antibody conjugate on the tumor cell surface, is unclear at the present time. We will report experimental results addressing this question in due course. Nevertheless, we think that the biotinylated 6 can serve as a model compound to explore the usefulness of non-radioactive small molecule

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Table 1: Cytotoxicity of compounds 5, 6, and doxorubicin against U937 leukemia cells in vitro

| Compd. | IC50 (nM) |
|--------|-----------|
| 5      | 0.09      |
| 6      | 0.7       |
| Doxorubicin | 100       |

Cells were incubated with drugs for 48 h and the experiments were performed according to our previously published method [26]; IC50 values are defined as the minimal drug concentration necessary to inhibit incorporation of [3H]thymidine by 50%, and are the averages of three experiments.

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Figure 1

Structure of CC-1065
anticancer drugs in the pretargeting strategy for cancer imaging and therapy.

Materials and methods

Method A

3-[(5-Amino-1H-indol-2'-yl)carbonyl]-1-(chlorom-ethyl)-5-hydroxy-1, 2-dihydro-3H-benz[e]indole (5).

A solution of CBI [27], 2, (15 mg, 76 µmol) in 3 mL of ethyl acetate saturated with anhydrous hydrogen chloride was stirred at room temperature for 30 min in the dark. The suspension was concentrated in vacuo to give intermediate 3. Without further purification, the latter was dissolved in DMF (0.5 mL) and treated with 5-nitroindole-2-carboxylic acid (18 mg, 87 µmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI, 43 mg, 225 µmol). The reaction mixture was stirred at room temperature overnight and purified by thin layer chromatography eluting with 50% hexane in ethyl acetate to give 4. Without further purification, the latter was dissolved in DMF (0.5 mL) and ethyl acetate (5 mL). The product was filtered, and the filter cake was hydrogenated for 1 h at ambient temperature under 1 atm pressure. The product was filtered, and the filter cake was washed with methanol (20 mL). The solvent was removed in vacuo, and ether was added. The solid was filtered, and washed to afford 5 (13 mg, 44% yield from 2). mp>300°C. 1H NMR (DMF-d7, ppm): 11.16 (brs, 1 H, NH), 10.46 (s, 1 H, OH), 8.24–6.81 (m, 9 H, Ar-H), 4.84–4.79 (dd, 1 H, J = 9.3, 11.1 Hz, N HH), 4.71–4.67 (m, 3 H, NCH H, NH2), 4.31–4.21 (m, 1 H, CH2ClC HCH2N), 4.79 (dd, 1 H, J = 9.3, 11.1 Hz, ClC HH), 3.93–3.88 (dd, 1 H, J = 7.9, 11.2 Hz, CH2CO), 1.70–1.30 (m, 6 H, (CH2)3CH2CO). HRMS calcd for C22H18ClN3O2 391.1088 found 391.1068.

3-[(5-Biotin-amino)-1H-indol-2'-yl]carbonyl]-1-(chloromethyl)-5-hydroxy-1, 2-dihydro-3H-benz[e]indole (6).

To a solution of 5 (2 mg, 5.1 µmol) in DMF (0.2 mL) was added N-hydroxysuccinimidobiotin (110 mg, 323 µmol), and the solution was stirred for 48 h at ambient temperature. The product was purified by thin layer chromatography eluting with ethyl acetate and methanol (4/1, v/v) to give 6 (1.5 mg, 48%) as a gray solid. 1H NMR (DMF-d7, ppm): 11.56 (brs, 1 H, NH), 10.55 (s, 1 H, OH), 9.91 (s, 1 H, NH), 8.25–7.21 (m, 9 H, Ar-H), 6.32 (s, 1 H, biotin NH), 6.24 (s, 1 H, biotin NH), 4.90–4.85 (t, 1 H, J = 11.0 Hz, N HH), 4.74–4.70 (dd, 1 H, J = 2.0, 11.0 Hz, NH H), 3.96–3.91 (dd, 1 H, J = 7.8, 11.1 Hz, CH HCl), 3.20–3.27 (m, 1 H, biotin H), 2.45–2.41 (t, 2 H, J = 7.7 Hz, COCH3), 1.8–1.5 (m, 6 H, CO(CH2)3). HRMS calcd for C32H32ClN5O4S (M + Na+) 640.1761, found 640.1759.

Method B

5-(Biotin-amino)indole-2-carboxylic acid (9)

To a solution of 7 (60 mg, 294 µmol) in DMF (1 mL) was added N-hydroxysuccinimidobiotin (110 mg, 323 µmol) and the solution was stirred overnight at room temperature. The product was purified by flash chromatography eluting first with ethyl acetate followed by acetone. The solvent was removed in vacuo. Without further purification, methanol (5 mL) was added followed by 1 N NaOH (2 mL). The reaction mixture was stirred overnight at room temperature. The precipitate was filtered, and the filtrate was neutralized using 20% HCl. The precipitate was filtered, and the filter cake was washed with water. The product was dried in air to afford 49 mg (41% yield from 7) of gray solid. 1H NMR (DMF-d6, ppm): 12.55 (brs, 1 H, COOH), 11.60 (s, 1 H, NH), 9.70 (s, 1 H, NH), 7.98–7.02 (m, 4 H, Ar-H), 6.39 (s, 1 H, biotin NH), 6.31 (s, 1 H, biotin NH), 4.33–4.29 (t, 1 H, J = 7.5 Hz, C4'-H), 4.17–4.13 (m, 1 H, C8'-H), 3.14–3.11 (m, 1 H, C5'-H), 2.85–2.81 (dd, 1 H, J = 5.2, 12.7 Hz, C7'-HH), 2.60–2.57 (d, 1 H, J = 12.1 Hz, C7'-H), 2.32–2.28 (t, 1 H, J = 7.4 Hz, CH3-CO), 1.70–1.30 (m, 6 H, (CH2)3CH2CO). HRMS calcd for C19H23N4O4S (M + H+) 403.1440 found 403.1435.
solvent was removed in vacuo, and ether was added. The solid was filtered, and washed with ether to give 17 mg (37% yield) of 6, whose NMR spectrum is identical to the product made using Method A.

Acknowledgment
We thank Jolande Murray for help with the manuscript. This work was supported in part by a grant from the National Institutes of Health (CA79357-01 to Y.W.).

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