DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF A NOVEL DZQ-POLYAMIDE CONJUGATE

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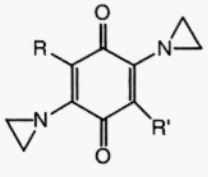
Abstract: This communication reports a practical synthesis of conjugate (**1**), which combines a netropsin analog and a 2,5-diaziridinylquinone moiety. Following continuous exposure, compound **1** was cytotoxic against the growth of human cancer cells in culture, with IC_{50} values in the range of 0.13 μM to 0.01 μM. It, however, displayed little differential cytotoxicity against cancer cells rich in DT-diaphorase [human lung cancer H460] and cells without the functional enzyme [human colon cancer BE].

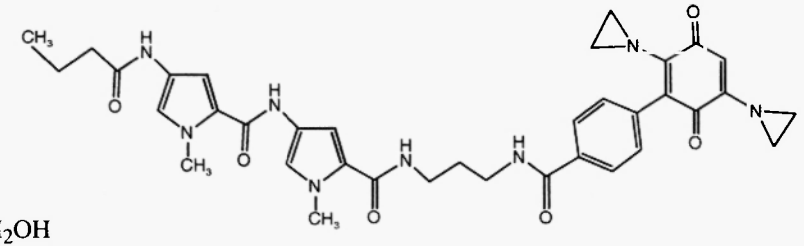
Introduction

DNA interactive agents belong to an important part of the chemotherapeutic arsenal for the treatment of cancer.[1] However, such cancer chemotherapeutic agents generally suffer from severe toxicity to the hosts, because they destroy both cancer and normal cells.[1] Consequently, in order to improve the therapeutic effectiveness of current anti-cancer drugs, there is an intense effort to rationally design compounds that will selectively kill cancer cells.[2]

The focus of this project centers on taking advantage of the general hypoxic nature of solid tumors.[3] Such tumors have environmens of low oxygen levels, and along with their higher levels of NADH and NADPH, they are conducive to promoting reduction reactions. DT-diaphorase, a reductive enzyme that uses either NADH or NADPH as a coenzyme, is present abundantly in some solid tumors.[4] For example, RH1, which contains a 2,5-diaziridinylquinone (DZQ) structure is preferentially reduced and activated by cancer cells that contain high levels of DT-diaphorase. The reduced form of RH1 reacts with guanine-N7 positions in the major groove to give interstrand crosslinks in the major groove.[5] Specifically, RH1 is 11,600 times more cytotoxic against H460 cells (DT-diaphorase containing) than BE cells, which contain a mutated and non-functional DT-diaphorase gene. RH1 is presently undergoing phase I clinical studies as an anticancer drug in the United Kingdom.[6]



RH1, R = CH_3, R' = CH_2OH
DZQ, R = R' = H



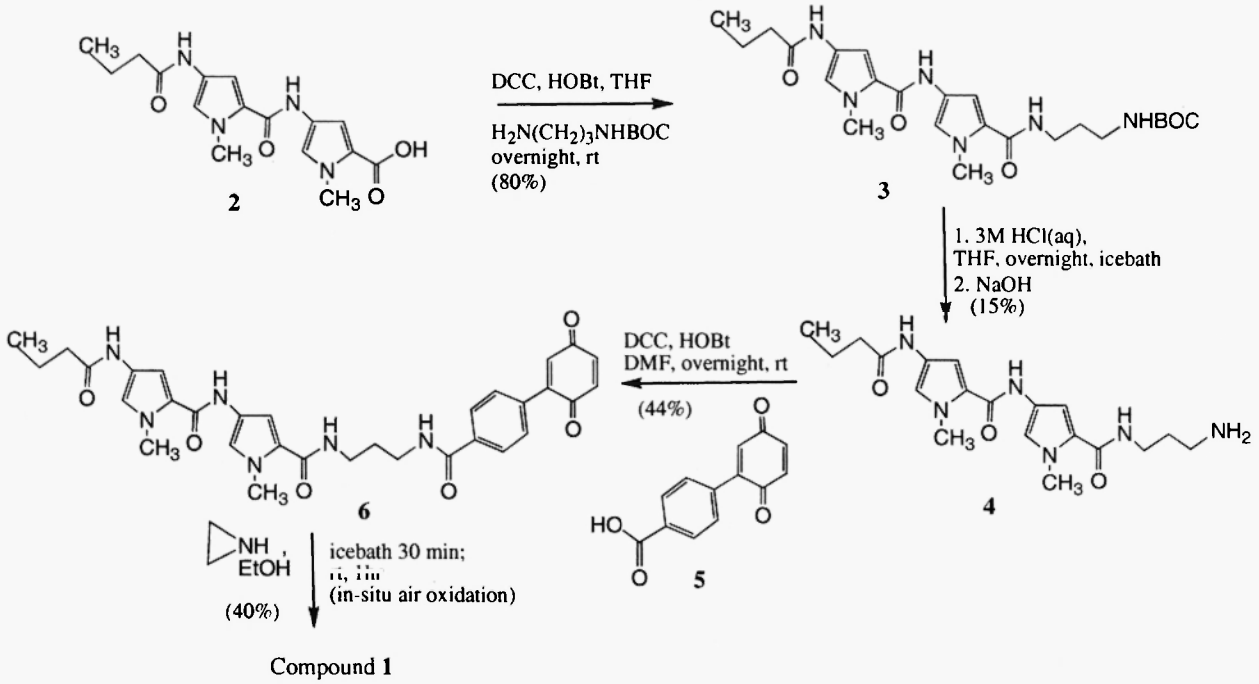
Compound **1**

In current developments of DNA interactive anticancer drugs, the minor groove has emerged as an attractive target. Minor groove and sequence selective agents that contain electrophilic functional groups have demonstrated potent anti-cancer activity. Examples of such agents include adozelesin and bizelesin, both analogs of CC-1065;[8] SJG-136, which is a dimer of two pyrrolobenzodiazepine units;[9] and tallimustine, a conjugate of distamycin with a benzoic acid mustard.[10]

In this communication, we report the synthesis of a conjugate of DZQ, a bioreductively activatable moiety, with a netropsin analog, an AT-sequence and minor groove selective binding agent. Upon synthesis of conjugate **1**, its ability to inhibit the growth of cancer cells with or without DT-diaphorase will be studied.

Results and Discussion

The synthetic strategy for the preparation of target conjugate **1** is outlined in Scheme 1. Beginning with the dipyrrole carboxylic acid intermediate **2**,[11] that our group had previously reported, it was coupled with N-BOC protected 1,3-diaminopropane in the presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) to give product **3** in 80 percent yield. Removal of the BOC protecting group by 3M hydrochloric acid, followed by a base work up gave the free primary amine **4** in 15 percent yield, after purification on a silica gel column. The low yield in this reaction was presumably due to acid hydrolysis of the amido groups in



Scheme 1. Synthesis of the target DZQ-polyamide conjugate **1**.

compound **3**, and decomposition of the resulting pyrrole-amines as indicated by the formation of highly polar and colored materials. Amine **4** was coupled to a quinone carboxylic acid **5**[12] in presence of DCC and HOBt to give compound **6** in 44 percent yield. The aziridinyl groups in conjugate **1** were introduced by reaction of compound **6** with aziridine, and the desired burgundy-colored solid product **1** was isolated in 40 percent yield after silica gel column chromatography. The structure of conjugate **1** and the intermediates were characterized by 500 MHz NMR, FT-IR, FAB-mass spectrometry and accurate mass measurements. Since aziridines tend to be reactive with nucleophiles, conjugate **1** was stored solvent-free and under an atmosphere of nitrogen in a dessicator that was kept inside a freezer.

The cytotoxicity studies were done using a MTT assay, and the cells were continuously treated with the compound for 5 days.[13] Two different human cancer cell lines were used for these studies. H460 is a human lung cancer cell line is rich in DT-diaphorase, but BE cells, that is derived from a human colon cancer, lack this bioreductive enzyme.[4] The results from the cytotoxicity studies, expressed as IC_{50} concentrations in M, are given in Table 1. For H460 cells, compound **1** was found to have comparable cytotoxic potency as RH1. However, unlike RH1 as indicated by the high selectivity index for H460 cells, compound **1** did not give a significant differential cytotoxicity against H460 cells over BE cells, indicating that DT-diaphorase promoted reduction is not required for conjugate **1** to exert its biological effect. It is possible that compound **1** and RH1 have different mechanisms activation, and studies to determine the mechanism of action of conjugate **1** is in progress.

Table 1. Cytotoxicity [IC_{50} values (M)] of conjugate **1** and RH1, following 5-day drug exposure.

Compound / Cells	H460	BE	BE/H460 (selectivity index)
Conjugate **1**	0.14 ± 0.01	0.015 ± 0.005	0.1
RH1	0.05	580	11600

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