Synthesis of Intermediates for Novel Cell- Specific DNA-damaging Agents for Cancer Therapy

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1. Introduction

Human DNA is susceptible to many damaging agents. When DNA-damage occurs, cells suspend progressing through the cell-cycle (cell-cycle arrest), and rely on repair enzymes to recognize and repair the DNA-damage in order to preserve normal cell functioning. However, on occasion, DNA repair is not possible. In these cases, cells maybe unable to enter into cell-cycle arrest (and therefore are unable to provide time for repair enzymes to repair the damaged DNA), or the repair enzymes themselves may be compromised. When cells continue through the cell cycle and begin to divide with the DNA damage still present, other enzymes notice the damaged DNA and initiate the destruction of the controlled destruction of these cells containing the damaged DNA.

Several clinically used DNA-alkylating (damaging) drugs use this mechanism to eradicate cancerous cells. These drugs damage the DNA of all cells. Most normal cells, after this damage to their DNA, go into cell cycle arrest and allow time for repair enzymes to fix the damage before the cell cycle continues and the cell starts dividing. However, cancer cells are rapidly dividing—they are incapable of going into cell cycle arrest. Therefore the DNA damage present in these cancer cells during their division triggers the other enzymes in these cells to kill these cancerous cells. Thus tumor cells are killed while normal cells survive the chemotherapy treatment.

However, some normal cells, such as hair cells and cells in the gastric lining, also divide rapidly. When the DNA in these cells are damaged during cancer chemotherapy, they too are unable to complete the repair of their damaged DNA before cell-division, and therefore succumb to the drug treatment. The destruction of these cells results in the side effects of hair loss, gastric irritation, etc., that are common in cancer chemotherapy treatments.

Another disadvantage of DNA-alkylating drugs currently used for cancer treatment is that they attack DNA at numerous sites; damage at some sites leads to the desired cell death, whereas damage at other sites leads to mutations instead, that can later develop into secondary cancers. In other words, drugs that are used to treat a primary cancer can "cause" a secondary cancer a few years after treatment. Also, the lack of specificity for cancer cells and the inability to cause only the kind of DNA damage that causes cell death results in significant wastage, and consequently in low potency of currently used DNA-damaging cancer drugs.

2. Objectives and Justification

There is an ongoing project in our lab trying to address these deficiencies by making new compounds that can target cancer cells and deliver only the kind of DNA damage that causes cell death. These new compounds could lead to the development of new cancer drugs that would have improved tumor-cell specificity, decreased side effects, minimized risks of secondary cancers, and increased potency.

Me-lex (**Figure 1)**[1], is a DNA-damaging compound that has been found to bind to specific sites in the minor groove of DNA and causes a specific type of damage that leads to cell death and does not lead to mutations[2]. Therefore, if Me-lex could be coupled with a cancer cell-targeting ligand, it could achieve the desired goals mentioned above.

Figure 1

The overall goal of an ongoing project in our laboratory is to synthesize compounds that would have the ability to target breast cancer cells while retaining the cytotoxicity of Me-lex. These compounds have the design shown in **Figure 3**. In this design, Me-lex is connected, via a linker, to estradiol, which is known to selectively target breast cancer cells[3,4]because it selectively binds to the estrogen receptor which is overexpressed (over-produced) in breast tumor cells.

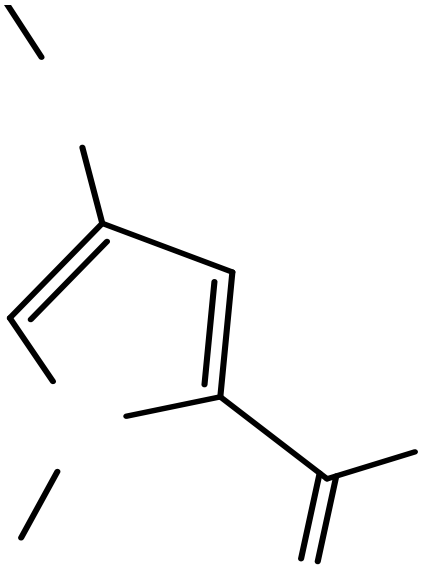
The goal of this project is to synthesize the DNA-binding section of this molecule, including three different linker units (**R** in **Figure 3**). The linker unit is expected to play an important role in optimizing both the DNA-binding and the cell-targeting properties of these compounds.

3. Methods

The DNA-binding section, containing three different lengths of carbon linkers (**R** = CH_2, CH_2CH_2, and $CH_2CH_2CH_2$) will be synthesized for this project. The overall scheme that will be used for preparing these compounds, starting from commercially available starting materials, is outlined in **Figure 4**. This synthetic scheme is based on previous publications[5] and upon experience from previously conducted experiments in the laboratory.

Each step of the reaction will be monitored to completion using Thin Layer Chromatography (TLC) analysis. Techniques such as suction filtration and rotary evaporation are used to isolate the products, and other techniques including liquid-liquid extractions, recrystallization, and flash column chromatography will be performed to purify the products. Nuclear Magnetic Resonance (NMR) and Infrared (IR) spectroscopy will be used to verify that the desired compound has been synthesized before continuing on to the next step. After the compound from each step of the synthesis has been isolated, purified, and characterized, it becomes the starting material for the next step of the synthesis, and this step-by-step procedure of isolation, purification, and identification is performed until the final product has been synthesized.

Once the compounds with the different linkers (3 different **R**s) have been synthesized, these compounds will be condensed with the estradiol cell-targeting ligand, and the reactive (DNA-damaging) methyl sulfonate unit will be introduced, as shown in **Figure 5**, in order to obtain the final desired compounds.



Future Work:

These final compounds will then be investigated with respect to their DNA-binding, DNA-damaging, estrogen receptor-binding, cell-targeting and selective cell-toxicity properties. These studies will enable us to determine the optimum length of the linker for binding, transport, specificity, and toxicity. These results from this study will be published in peer-reviewed journals, and would aid in the design of drugs that can selectively destroy cancer cells without causing secondary cancer while minimizing side-effects.

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