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Synthesis and preliminary evaluation steroidal antiestrogen–geldanamycin conjugates

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ABSTRACT

Three novel steroidal antiestrogen–geldanamycin conjugates were prepared using a convergent strategy. The antiestrogenic component utilized the 11β-(4-functionalized-oxyphenyl) estradiol scaffold, while the geldanamycin component was derived by replacement of the 17-methoxy group with an appropriately functionalized amine. Ligation was achieved in high yield using azide alkyne cyclization reactions. Evaluation of the products against two breast cancer cell lines indicated that the conjugates retained significant antiproliferative activity.

Introduction

Breast cancer is the most prevalent form of cancer in women and the well-established association between the human estrogen receptor (ER) and cell proliferation provided the basis for endocrine (antihormonal) therapy.[1,2] However, prolonged treatment with antiestrogens often results in the development of hormonal resistance, leading to recurrence of the disease and the use of more potent, but nonselective, therapeutic agents.[3–5] One strategy that attempts to circumvent the effects of resistance is the use of drug conjugates in which two therapeutic agents are combined into a single entity.[6–8]

As part of our program in breast cancer research, we have focused on using ER as a targeting mechanism for which the steroidal anti-estrogenic component may also provide a beneficial therapeutic response. The choice of the therapeutic component is also critical as it should not only be active within the same concentration range as the hormonal component but exert a complementary or synergistic effect. The ER-targeting component was developed in our initial work with the 11β-(4-substituted-oxyphenyl) estradiols.[9,10] Based on the affinity of the steroids for the ER and their antiestrogenic activity, we prepared a steroidal antiestrogen–mitomycin C conjugate to test our concept.[11] Although the compound retained high ER affinity and antiestrogenic properties, it was no more active than mitomycin C and displayed no selectivity toward ER-expressing breast cancer cells. One possible explanation for the lack of synergy may have involved the properties of the linker. Unfortunately, issues regarding the availability mitomycin C precluded further studies with this conjugate. Therefore we elected to evaluate the effect of linker length and conformational flexibility using the Hsp90 N-terminal inhibitor, geldanamycin (GDA), as the therapeutic component (Fig. 1).

Heat shock proteins (HSP) are molecular chaperones that are critical for the maintenance of cellular homeostasis through regulation of protein transport, conformational folding and maturation.[12] Hsp90 is a 90 kDa protein that is often overexpressed in breast cancer, as well as other cancers, and, as a result of these increased levels, is responsible for maintaining high levels of active oncogenic proteins.[13–15] One of these proteins is ERα which, when dormant, is confined to the nucleus in an Hsp90 complex.[16] Disruption of the Hsp90-ERα complex leads to improper folding of ERα and its subsequent degradation, resulting in down-regulation of its corresponding pathways, such as transcription. Therefore, disruption of Hsp90-mediated responses provides an alternative target for breast cancer therapy, and has led to the use of geldanamycin (GDA) and its derivatives as therapeutic agents.

The geldanamycin component was developed based upon our work with chaperone inhibiting agents. Structure–activity relationship studies demonstrated that modification at the 17-position not only generates GDA derivatives that exhibit reduced toxicity, but this position is also substituent tolerant as groups at this position of GDA exit the Hsp90 binding pocket and thus do not significantly affect inhibitory activity.[17] Other 17-GDA derivatives have been synthesized that exhibit improved solubility and lower toxicity than GDA, but are still hepatotoxic.[18,19] Therefore we planned to

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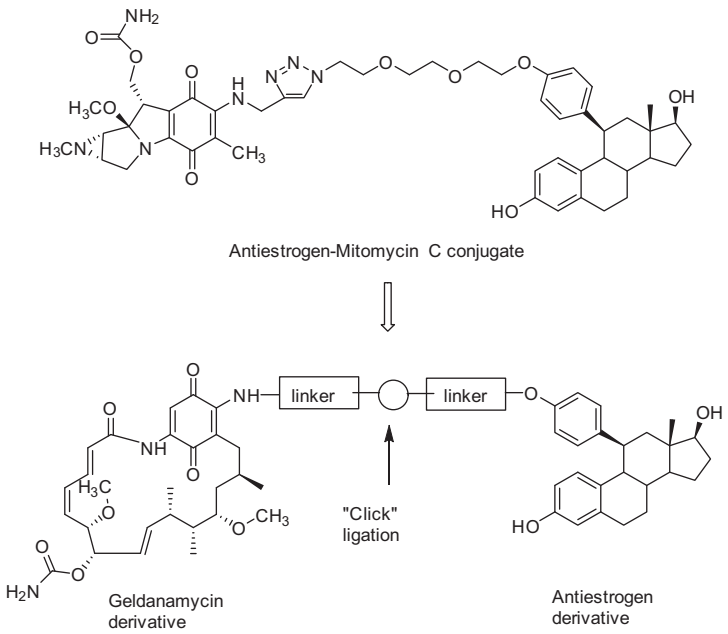

Figure 1. Proposed extension of research from the antiestrogen–mitomycin C conjugate to the antiestrogen–geldanamycin conjugates.

introduce modifications at the 17-postion that will permit conjugation to the steroidal derivatives.

We chose a convergent approach in which each component contained a side chain that is terminally substituted with a reactive functionality. The final step then involves a ligation reaction under mild conditions. The reaction selected for this study was the Huisgen [3+2] cycloaddition reaction between a terminal azide and a terminal alkyne to generate a chemically stable triazole moiety.[20–22] The reaction has the advantage of being chemoselective and allowing the reactive groups to reside on either component. In this study we chose to use different lengths of the linker to investigate what effect, if any, it exerts on the biological activity of the final conjugate. The overall synthetic strategy for our conjugates is shown in Figure 2.

Results

The synthesis of the steroidal antiestrogen component was accomplished using a strategy similar to one described for our 11β-(4-substituted oxyphenyl) estradiols.[9,10] Scheme 1 deltenone 3-ethylene ketal **1** was converted initially to the 11β-(4-hydroxyphenyl) estra-4,9-diene-3,17-dione **2**. This compound then served as the intermediate for the preparation of the requisite 11β-(4-azidoethoxyphenyl)estradiol **4a** and 11β-(4-*N*-propargyl-*N*-methylaminoethoxyphenyl) estradiol components **4b**. For the propargyl derivative, we prepared the 2-(*N*-propargyl-*N*-methylamino)ethanol which was then coupled to the 11β-(4-hydroxyphenyl) estra-4,9-diene-3,17-dione **2** using the Mitsunobu reaction to give **3b**. Aromatization with acetic anhydride-acetyl bromide followed by reduction–saponification gave the desired product **4b**. Overall yields for the two compounds were 28% (eight steps) and 19% (seven steps), respectively. We had previously characterized the azido derivative **4a**, determined its binding affinity (RBA = 39%) and showed that it was a full antagonist of ERα. The *N*-propargyl-*N*-methyl derivative **4b** is a close analog of the RU39411 for which we had determined ER affinity (RBA = 39%) and efficacy (full antagonism). Having demonstrated that additional substituents distal to the nitrogen in the side chain did not adversely affect either binding

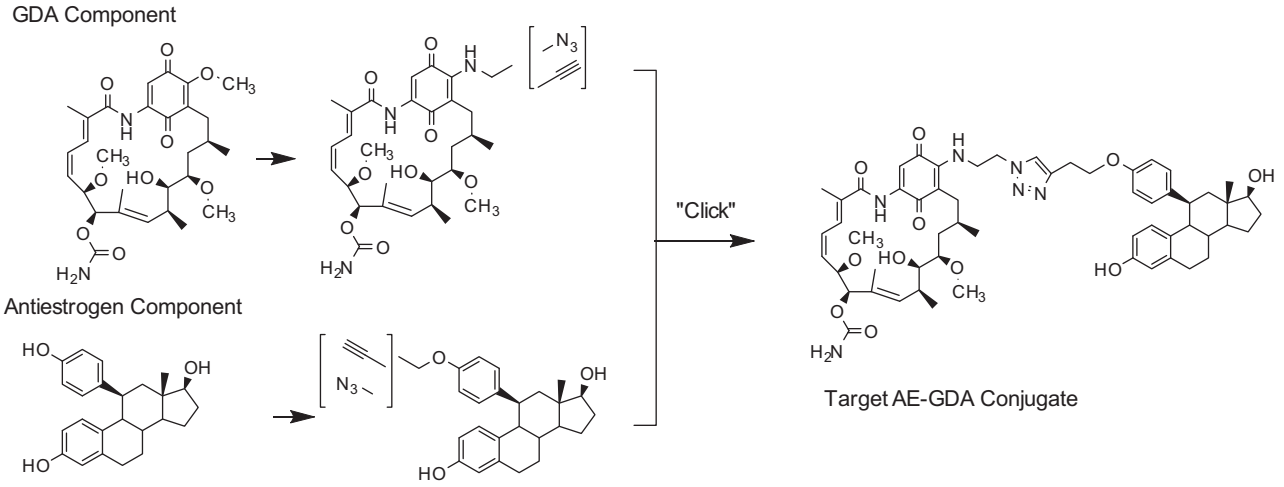

Figure 2. Approach for synthesis of individual components and assembly as AE–GDA conjugates.