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Synthesis and Evaluation of Geldanamycin–Testosterone Hybrids

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Abstract—Geldanamycin (GDM) binds to the Hsp90 chaperone protein resulting in the degradation of several important signaling proteins. A series of GDM–testosterone linked hybrids has been synthesized and evaluated for activity against prostate cancer cell lines. The hybrid with the greatest activity exhibits potent and selective cytotoxicity against prostate cancer cells containing the androgen receptor.

Introduction

Geldanamycin[1] (GDM), first isolated from *Streptomyces hygroscopicus*, was originally identified as a potent inhibitor of cells transformed by the v-src oncogene.[2] It was later shown that GDM did not directly inhibit the src-encoded tyrosine kinase, but rather caused the proteosome dependent degradation of c-src and certain other signaling proteins.[2,3] GDM exerts its biological effects by binding to the highly conserved N-terminal ATP binding pocket of the molecular chaperone Hsp90.[4–7]

Hsp90[8] is an abundant chaperone protein that plays a role in the process of protein refolding and in the conformational maturation of several signaling molecules,[9] including steroid receptors,[10] Raf protein kinase,[11] and several transmembrane tyrosine kinases.[12,13] Occupancy of the Hsp90 pocket by GDM leads to the proteasomal degradation of these proteins and resultant cell death. GDM causes growth arrest and subsequent apoptosis of cancer cells and a related analogue is currently in early clinical trial.[14] However, the broad spectrum of activity of GDM against multiple important regulatory proteins suggests that its medicinal use will be limited by significant toxicity. As a result, we have initiated a program aimed at synthesizing and identifying derivatives of GDM that selectively inhibit or degrade particular proteins that are necessary for cancer growth.

We have previously shown that an appropriately fashioned hybrid that links estradiol (E2) and GDM (Fig. 1, below) demonstrated selective degradation of the estrogen receptor (ER) and HER2 kinase in MCF7 breast cancer cells, while having diminished effects against Raf-1 and IGF1R.[15] Our goal was to synthesize a GDM derivative, with less toxicity than GDM, which would be selectively cytotoxic toward breast cancer cells that express ER.

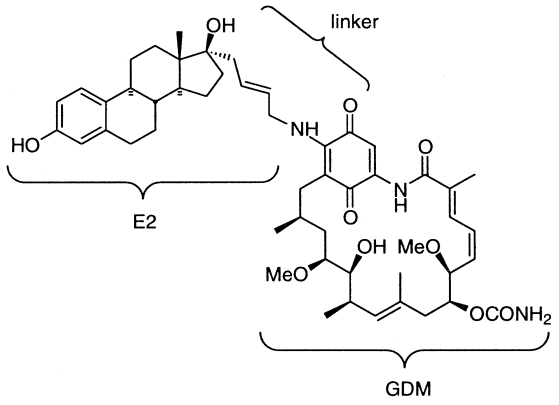

Figure 1. GDM-estradiol (E2) heterodimer that displays selective degradation of target cells containing ER.

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Since the AR is required for the unregulated growth of prostate carcinomas and tumor progression to an advanced, hormone-independent state is often accompanied by AR mutation, a compound that induces the selective degradation of the AR could be a useful therapeutic agent. In this report, we describe the synthesis of GDM–testosterone hybrids designed to selectively inhibit the androgen receptor.

Chemistry

The general strategy for linking GDM and testosterone is shown in Figure 2. Although limited SAR data is available for testosterone compared to E2, the C17-β-hydroxy group is still required for strong binding affinity.[16] Our strategy centered on the α-stereoselective addition of a lithiated alkynol to the C17-ketone of testosterone.[17] The hydroxyl could then be elaborated to a terminal primary amino group for coupling to GDM. Our laboratory[12] and others[18] have reported that the C17 methoxy of the benzoquinone undergoes smooth Michael-like reaction with amines. Previous SAR work[15] and a recent GDM-Hsp90 crystal structure[4] show that C17 of GDM is tolerant to a variety of substitution patterns.

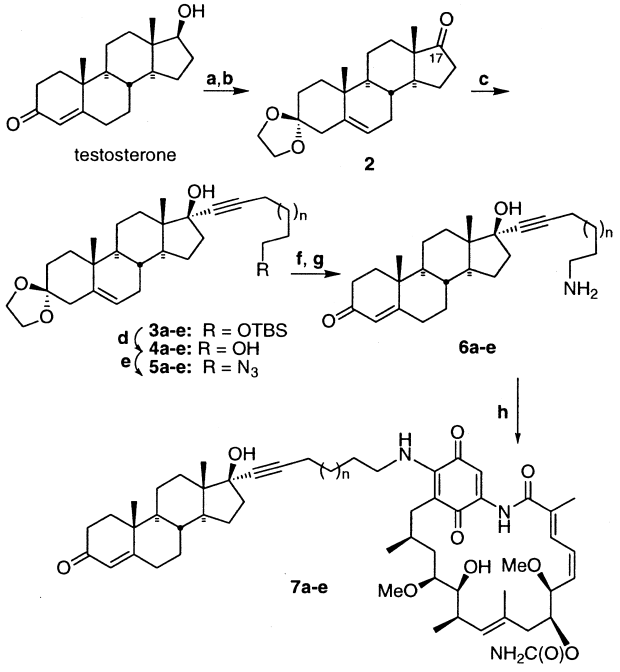
In the event, testosterone was protected as its C3 cyclic ketal derivative. The ketalization step was accompanied by olefin migration to C5 (Scheme 1). Subsequent TPAP oxidation at C17 afforded ketone **2**. The addition of excess lithiated *t*-butyldimethylsilylalkynyl ether to the C17-ketone gave quantitative yields of the desired products, **3a**–**e**,[19] with exclusive addition from the α-face. Treatment of this product with TBAF followed by a three-step sequence afforded good yields of the azide, **5a**–**e**. Removal of the ketal with 1 N HCl in MeOH, also resulted in re-conjugation of the olefin. Finally, the azide was reduced with PEt_3 to afford the amines, **6a**–**e**, which were used immediately in the coupling with GDM. The hybrids **7a**–**e** were obtained in 75–100% yields.

The alkyne of the carbon linker was also modified as shown in Scheme 2, below. Conversion to the *E*-olefin was accomplished using LAH in THF to afford **8a**, while Lindlar reduction produced *Z*-olefin **8b**. Full saturation to the alkane without reducing the ring olefin was accomplished with Wilkinson's catalyst as demonstrated for substrate **8c**. The alcohols were then converted to desired hybrids **9a**–**c** as described in Scheme 1.

Biological Results

As part of our initial study hybrids **7a**–**e**, **9a**–**c**, and GDM were first evaluated for cytotoxicity against LNCaP prostate cancer cells as shown in Table 1.

A very clear SAR pattern based upon the size and nature of the linker can be discerned. The 6-carbon alkynyl hybrid, **7c**, is the most potent with an IC_{50} of 100 nM compared to the parent GDM at 40 nM. The 5-carbon alkynyl linker is the next potent at 200 nM, however,



Scheme 1. (a) *p*-TSA, $(CH_2OH)_2$, heat, 80%; (b) TPAP, NMO, CH_2Cl_2, quantity; (c) TBS-alkynol, *n*-BuLi, pentane-THF, 0 °C, quantity; (d) TBAF, THF, 0 °C, quant.; (e) i. MsCl, TEA, CH_2Cl_2, ii. NaN_3, DMF; (f) 1N HCl, MeOH; (g) PEt_3, THF, H_2O; (h) GDM, DMSO, 2 h, 75–100% yield.

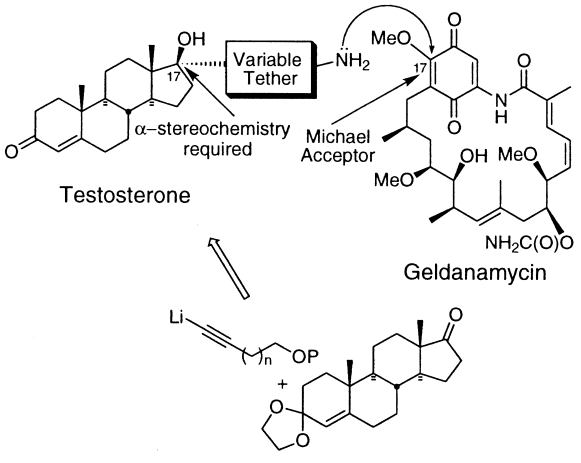
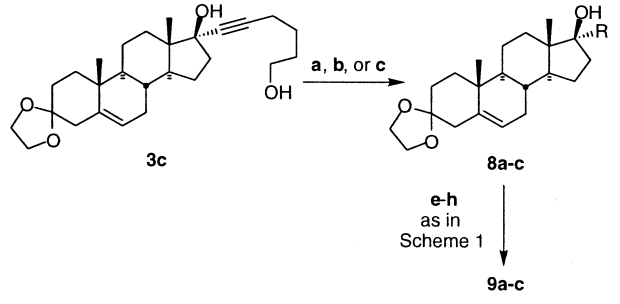
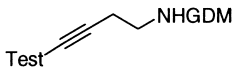
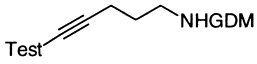
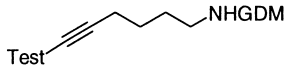
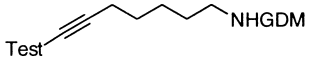
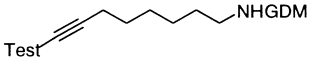
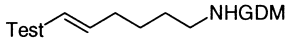
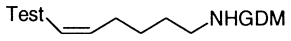
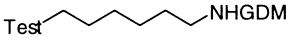


Figure 2. Structures and strategy for hybridization of testosterone and geldanamycin.



Scheme 2. (a) LAH, THF, reflux; (b) $Pd/CaCO_3$, EtOH, H_2, quinoline; (c) $RhCl(PPh_3)_3$, EtOAc, H_2.

Table 1. Cytotoxicity for GDM and new hybrids **7a–e** and **9a–c** in LNCaP prostate cancer cells

Compound	Linker	IC_{50}
GDM	--------	40 nM
7a		>1 μM
7b		200 nM
7c		100 nM
7d		>1 μM
7e		>1 μM
9a		>1 μM
9b		>1 μM
9c		>1 μM

shorter or longer tether lengths are less potent, all greater than 1 μM in potency. Further analysis of the alkyne functionality in the 6-carbon linker shows that it is absolutely crucial for potency. Reduction to the *E*-(**9a**) and *Z*-(**9b**) olefins, (or to the fully saturated system, **9c**) all result in complete abrogation of activity. We have previously observed the importance of the linker element on activity in the E2-GDM series as well, although the attachment in that series was at the C-16 position of E2.[15]

Table 2 compares the IC_{50}'s of GDM and **7c** on three prostate (two AR-dependent and one AR-independent), two breast (one ER-dependent and one ER-independent), and one colon (ER- and AR-independent) cancer cell line. In this experiment, both GDM and **7c** were potent against the LNCaP (mutated AR) and LAPC4 (wild-type AR) cell lines. However, most striking is the activity against the AR-independent PC3 cells, in which GDM was still very potent, but the hybrid structure **7c** *was 13 times weaker*. A similar trend was observed with MCF7 breast cancer cells (AR-independent), wherein **7c** was very weak ($IC_{50} = 300$ nM) while GDM was a potent inhibitor ($IC_{50} = 30$ nM).

Two important deductions can be drawn from the IC_{50} data. First, attachment of testosterone to GDM affords a compound that exhibits selected cytotoxicity for cells which are AR-dependent, demonstrating the success in our strategy of selectively targeting AR-dependent cells. Second, in AR-dependent cells, there is only a slight attenuation of activity of the hybrid compound **7c** compared to GDM. This is a particularly exciting result when one takes into account the attachment of the considerably large testosterone structure to the GDM nucleus.

Table 2. Enhanced cytotoxicity of the GDM and **7c** towards AR-dependent cells

Cell line	Origin	GDM IC_{50} (nM)	**7c** IC_{50} (nM)
LNCaP	Prostate	40	100
LAPC4	Prostate	25	40
PC3	Prostate	20	300
MCF7	Breast	30	300
MDA468	Breast	20	240
Colo205	Colon	20	200

The mechanism whereby the hybrid molecule, **7c**, selectively inhibits the growth of cancer cells that are AR dependent is currently under investigation. Preliminary data (not shown) suggests that **7c** is much less active than GDM in inducing degradation of the AR and other cellular proteins, but that it may inhibit AR function by causing the cytoplasmic sequestration (data not shown) of AR. These findings and the demonstrated selective inhibition of cell growth, suggest that **7c** may be less toxic than GDM while still retaining activity against prostate cancer. Furthermore, both GDM and **7c** induce the degradation of wild-type (LAPC4) and mutated (LNCaP) androgen receptor.[20] This finding suggests that the hybrid (**7c**) might be useful in the considerable portion of advanced stage, hormone-independent prostate cancers that express the mutated, activated form of AR.

Conclusion

We have designed and prepared a series of C17-C17 testosterone–GDM linked hybrids. While the potency of GDM is independent of the presence or absence of AR, one of our newly synthesized hybrid molecules, **7c**, exhibits strong and selective cytotoxicity towards prostate cancer cells expressing AR. Our current research efforts are centered toward determination of the mechanism of selective inhibition of AR by the GDM hybrid, **7c**.

We are also investigating the efficacy of the hybrid compound, **7c**, in an animal model to determine if the selectivity can be realized in vivo.

Acknowledgements

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