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SYNTHESIS AND EVALUATION OF GELDANAMYCIN-ESTRADIOL HYBRIDS

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Abstract: Geldanamycin (GDM) binds to the Hsp90 chaperone protein and causes the degradation of several important signalling proteins. A series of novel estradiol-geldanamycin hybrids has been synthesized and evaluated for their ability to induce the selective degradation of the estrogen receptor (ER). The hybrid compounds are active and more selective than the parent causing degradation of ER and HER2, but not other GDM targets.

Introduction

The ansamycin antibiotic Geldanamycin[1] (GDM), first isolated from *Streptomyces hygroscopicus*, was originally identified as a potent inhibitor of src kinase.[2] Later work showed that GDM was not directly an inhibitor of this kinase, but that it inhibited kinase activity by inducing the proteosome dependent degradation of Src and other tyrosine kinases.[2,3] The ansamycins exert their biological effects by binding to a highly conserved pocket in the molecular chaperone Hsp90.[4-6] The latter is an abundant chaperone protein, required for protein refolding after stress and for the conformational maturation of some signalling molecules. Ansamycins inhibit these processes and induce the degradation of Hsp90 substrates.[7-9] The range of signalling proteins affected by GDM is expected to result in non-selective toxicity thereby compromising its applicability as a therapeutic agent. We have begun a program to prepare derivatives of GDM that would selectively induce the degradation of particular proteins.

In this connection, it was hoped that an appropriately fashioned hybrid drug linking estradiol (E2) and GDM would show higher affinity for estrogen receptor (ER). Our initial goal here was to first design and synthesize hybrid compounds and then check for activity in comparison to the parent GDM. The ultimate goal was to realize selectivity in the degradation of specific proteins. Particular targets might be the ER and HER2. HER2 is a transmembrane kinase that is amplified and overexpressed in a significant number of breast cancers. Successful targeting of these proteins could lead to arrested cell growth and/or apoptosis. We describe herein our preliminary results in synthesizing these compounds and resultant biological data.

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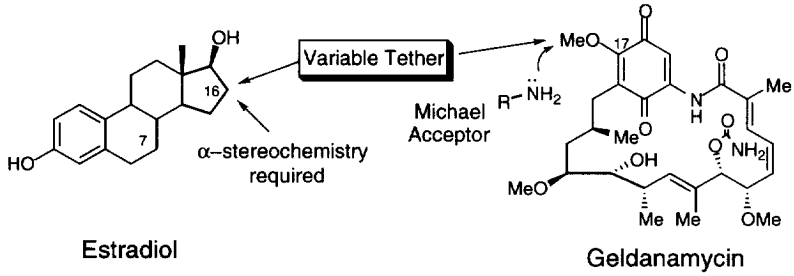
Our initial goal was to investigate the linkage on E2 and GDM in a manner that would allow for retention of activity for both components. Concerning the chemical linkage of the compounds, the site we investigated on estradiol[10] was the C-16[11] position. For high ER binding affinity the stereochemistry at C16 should be α.[12] As regard to GDM, our laboratory[9] and others[13] had reported that the C-17 methoxy of the benzoquinone undergoes smooth Michael-like reaction with amines. SAR data[13] has also shown C-17 to be tolerant to substitution and this was confirmed by a GDM-Hsp90 crystal structure.[4] Therefore, we required a strategy in which a linker element had to be constructed in an α-stereoselective fashion at C-16 of estradiol, with the provision that it could further be elaborated to a terminal primary amino group for coupling to GDM.

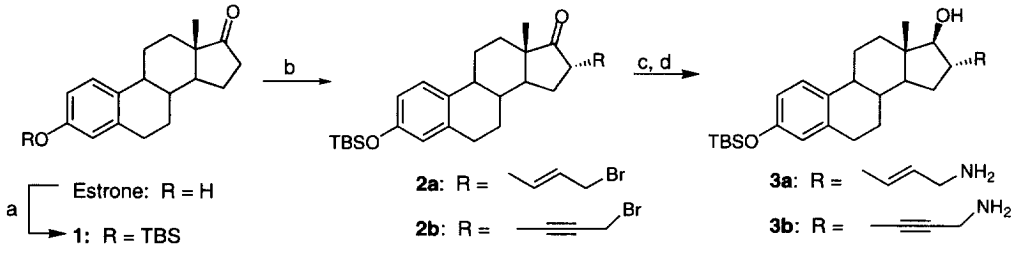
Figure 1. Structures and strategy for hybridization of estradiol and geldanamycin.



Chemistry

For connection at the C-16 position, a potentially problematic[14] α-alkylation of the C-17 ketone of estrone would be required. Our initial attempts to achieve this result via the C17 ketone enolate of TBS protected estrone **1** and unactivated electrophiles (alkyl bromides/iodides) resulted in poor yields, bis-alkylation, and predominantly β-products. However, Katzenellenbogen's method[15] using (*E*)-1,4-di-bromo-2-butene, 1,4-di-bromo-2-butyne, allylbromide, and carefully controlled temperatures, gave exclusively the desired α-alkylation products **2a-c** in fair to good yields. Following conversion of the resulting bromides to the azides by sodium azide, stereoselective reduction of the C-17 ketone at -78 °C followed by warming to -10 °C to reduce the azide, produced amino alcohols **3a** and **3b** in excellent yields.

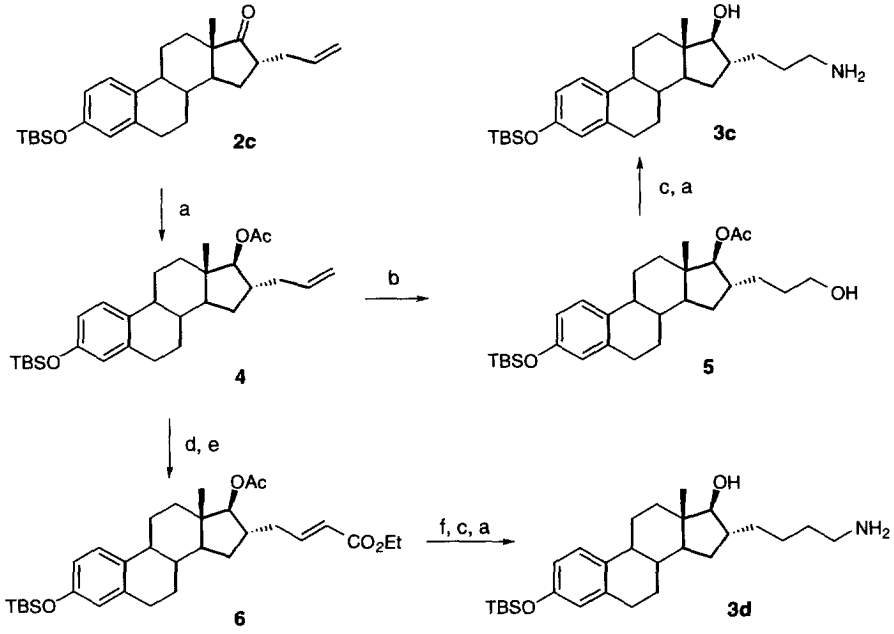
Scheme 1



Reagents: (a) TBSCl, imidazole, DMF, 86%; (b) i) LDA, THF, 0 °C, ii) electrophile, -35 °C, 12 h, 40-75%; (c) NaN_3, DMSO, THF, H_2O, 80-100%; (d) LAH, THF, -78 °C to -10 °C, 3 h, 100%.;

Again since alkyl halides were poor electrophiles, the allylated precursor **2c** was used as a handle for elaboration to prepare 3- and 4- carbon saturated linkers with the desired C-16α configuration (Scheme 2). Following selective LAH reduction to the C-17β alcohol, protection as the acetate afforded **4**. Hydroboration/sodium perborate oxidation of **4** afforded 3-carbon alcohol **5**. Conversion of **5** to the azide via the mesylate and subsequent LAH reduction of the azide and C-17 acetate provided amino alcohol **3c**. For the 4-carbon saturated linker, oxidative cleavage of **2c** with $OsO_4/NaIO_4$ followed by Horner-Emmons homologation and hydrogenation of the resulting olefin afforded **6** in 95% yield. Reduction of the esters and subsequent conversion of the primary alcohol to the amine afforded the fully saturated 4-carbon linker **3d**.

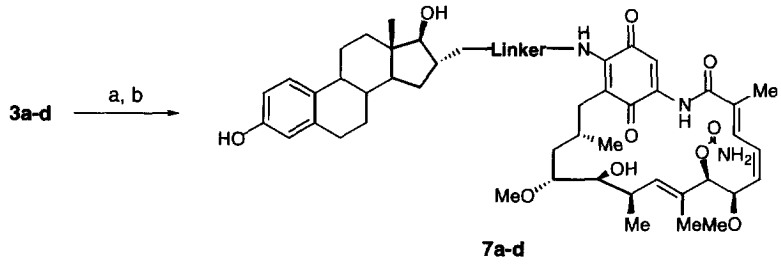
Scheme 2



Reagents: (a) i. LAH, THF, -78 °C to -10 °C, 3 hrs, quant. ii. Ac_2O, DMAP, CH_2Cl_2, 95%; (b) i. 9-BBN, THF, ii. $NaBO_3$, H_2O, 70%; (c) i. MsCl, Pyr, ii. NaN_3, DMF, 80%; (d) i. OsO_4, NMO, ii. $NaIO_4$, THF; (e) $Ph_3P{=}CHCO_2Et$, PhH; (f) i. Pd/C, H_2, EtOAc, 3 steps-95%, ii. LAH, THF, 0 °C

For coupling to GDM, we relied on the aforementioned Michael behavior at the C-17 position with primary amines.[16] In the coupling event, amino-alcohols **3a-d** underwent smooth reaction with GDM in DMSO overnight, followed by deprotection of the phenolic TBS ether with TBAF-HOAc to afford estradiol-GMD hydrids **7a-d** cleanly in fair to good yields (40-60%). The coupling proves very clean and unreacted GDM can be recovered from the reaction mixture. The use of an excess (2.0 equiv.) of amino alcohols **3** gave much higher yields of **7a-d** (>85%).

Scheme 3



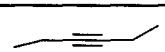
Reagents: (a) Geldanamycin, DMSO; (b) TBAF-HOAc, THF.

Biological Evaluation of Constructs

For initial evaluation, the effects of GDM and **7a-d** on the steady-state levels of HER2, ER, and Raf-1 in MCF7 breast cancer cells were measured. The activity of was quantitated by determining the concentration of each compound needed to reduce the protein expression by 50% (i.e. the IC_{50}-HER2).[17] Results are shown in Table 1.

GDM was, as expected, the most active against all three proteins, particularly HER2 and ER. It is also clear that the hybrid drugs do indeed retain activity. The extent of this proves to be very strongly dependent on the nature of the tether or linker between the estradiol and GDM. For example, analog **7a** with the *E*-2-butene linkage, is the most active of the series. Going from an alkene to an alkyne in the linker for **7b** results in almost identical activity (except against ER where a slight decrease was noted). Most dramatic is the effect of saturation of the linker or shortening to 3-carbons in analogs **7c** and **7d**. For these two compounds, all activity is essentially lost. Thus, the activity of the constructs is surprisingly sensitive to the nature of the unsaturation in the linker element.

Table 1. The effects of GDM and **7a-7d** on steady state levels of HER2, ER, and Raf-1 in MCF7.

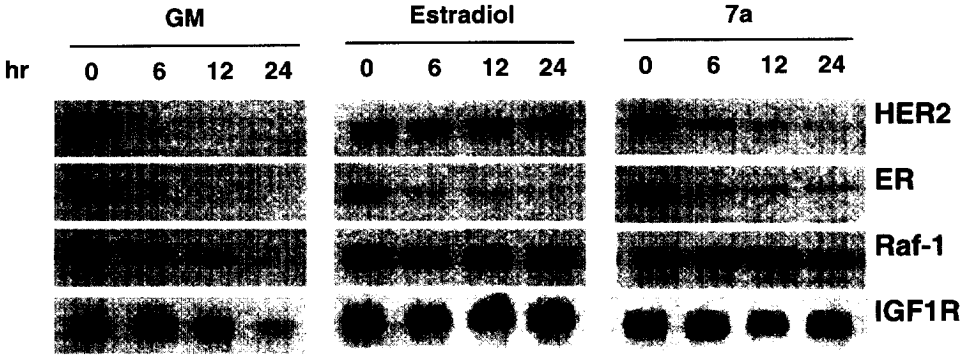
Compound	Linker	IC_{50}-HER2 (μM)	IC_{50}-ER (μM)	IC_{50}-Raf (μM)
GDM	-	0.05	0.06	0.2
7a		0.1	0.08	1.5
7b		0.1	0.22	1.5
7c		>2000	>2000	>2000
7d		>2000	>2000	>2000

Since these new hybrids do exhibit activity, a comparison of the effects of GDM, estradiol, and construct **7a** on HER2, ER, Raf-1, and IGF1R was made in MCF7 cells. The results are shown in Figure 2.[18]

GDM causes rapid degradation of HER2 and ER after 6 hr, and Raf-1 and IGF1R after a slightly longer time period. E2 is known to induce the downregulation of its own receptor.[19] E2 and **7a** both cause downregulation of ER in MCF7. As expected, treatment of MCF7 cells with E2 affected the steady state levels of ER, while having no effect on the other proteins. The effects of E2 on ER are complex and involve an interaction with the ER promoter. However, E2 stimulates breast cancer cell growth and **7a** selectively inhibits the growth of ER-containing cells.[20] The data suggest that induction of degradation of ER by **7a** is not preceded by receptor activation. Lead compound **7a** however, still exhibits very good activity against HER2 and ER, albeit somewhat attenuated compared to GDM.

Most remarkable is the fact that **7a** has essentially no effect on IGF1R and reduced activity against Raf-1[21] indicating that hybridization of GDM and estradiol does indeed exhibit selectivity. That is **7a** appears to be targeting the ER-Hsp90 interaction exclusively leaving other Hsp90 associated proteins unaffected. This could lead to an improved therapeutic profile compared to GDM. For example, Raf-1 represents an important intermediate of several transduction pathways. GDM analogs, with reduced activity against Raf-1, are desirable as they may prove to be less toxic to normal cells. In addition, in a prostate cancer cell line, GDM induced the loss of androgen receptor; **7a** had no effect confirming its specificity.[20]

Figure 2. The effects of GDM, E2, and **7a** on the steady state levels of HER2, ER, Raf-1, and IGF1R.



We note that these same linkers have previously been reported for attaching fluorescent labels/radiotracers to the C-16α position as well as to other estradiol derivatives.[22] The binding affinity of these constructs was also strongly dependent upon the nature of the tether, with the four carbon unsaturated ligands (corresponding to **7a** and **7b**) showing the highest degree of binding for ER. It is therefore likely that the activity of our compounds is a consequence of binding to ER otherwise the role of the linker should not play such an important effect. Further experiments to investigate these hypotheses, *in vivo* models, and optimization of hybrid structure are actively underway.

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16. We have observed that primary amines give a faster reaction that secondary ones, with the exception being secondary amines constrained in a ring (3-6 membered).
17. MCF-7 cells were treated with various concentrations of GDM or **7a - d** for 24 h. The same amounts of cell lysates were separated by electrophoresis. Steady state levels of HER2, ER, and Raf-1 were measure by immuno-blotting with specific antibodies, quantitated using Gel Doc 1000 (Bio-Rad). IC_{50} is the amount of each protein to inhibit protein expression by 50% compared to the control.
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