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Synthesis of potential allosteric modulators of Hsp90 by chemical glycosylation of *Eupomatenoid-6*

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ABSTRACT

Hsp90 (Heat shock protein-90) is a chaperone protein and an established anti-apoptotic target in cancer therapy. Most of the known small-molecule inhibitors that have shown potent antitumor activity target the Hsp90 N-terminal domain and directly inhibit its ATP-ase activity. Many of these molecules display important secondary effects. A different approach to Hsp90 inhibition consists of targeting the protein C-terminal domain (CTD) and modulating its chaperone activity through allosteric effects. Using an original computational approach, allosteric hot-spots in the CTD have been recently identified that control interdomain communication. A combination of virtual and experimental screening enabled identification of a rhamnosylated benzofuran (*Eupomatenoid-2*) as a lead for further development. In this paper we describe glycodiversification of *Eupomatenoid-2* using chemical glycosylation of the 2-(4′-hydroxyphenyl)benzofuran aglycon (a.k.a. *Eupomatenoid-6*). Glycosylation of the phenol by glycosyl bromides under basic conditions afforded the desired products in the *gluco*-, *galacto*-, and *fuco*-series. This approach failed in the *manno*- and *rhamno*-series. However, mannosylation and rhamnosylation of *Eupomatenoid-6* could be obtained under carefully controlled acidic conditions, using *O*-benzoxazolyl imidate (OBox) donors. The glycosides obtained are currently under investigation as modulators of Hsp90 chaperone activity.

1. Introduction

Heat Shock Proteins (HSPs) are a class of functionally related chaperone proteins, which are overexpressed as a protective mechanism in cells exposed to a variety of stressful events. They have been shown to possess a pivotal role in cell cycle progression and cell death (apoptosis) and to be involved in many diseases. In particular, Hsp90 is established as an anti-apoptotic target in cancer therapy.[1,2] Hsp90 consists of four domains, an N-terminal ATP binding site domain, a middle domain that regulates ATP hydrolysis, a charged region, and a C-terminal homodimerization domain.[3] These domains are involved in complex internal dynamic processes that control the chaperone activity of the protein and, with it, the signaling pathways regulated by Hsp90, which controls a number of client proteins.[1,4]

Numerous small-molecule inhibitors of Hsp90 have been identified and have shown potent antitumor activity in a wide-range of malignancies.[5] Most of these molecules, such as Geldanamycin or Radicicol,[6] target the Hsp90 N-terminal domain and directly inhibit its ATP-ase activity. The aminocoumarin Novobiocin and its analogues[7,8] have been shown to bind the C-terminal domain (CTD) of Hsp90 and to cause proteosomal degradation of the clients by inhibiting correct folding. A recent study has shown that the activity and selectivity of Novobiocin can be tuned by glycosylation at the 4′ position.[9]

Using an original computational approach,[10,11] allosteric hot-spots in Hsp90 CTD that control interdomain communication have been recently identified by Colombo and co-workers. Virtual screening enabled the identification of a group of 14 molecules targeted to these hot spots, six of which were experimentally shown to bind the CTD and to control Hsp90 function in cellular studies.[11] Allosteric modulation of Hsp90 may allow adjustments to the internal dynamics of the protein, leading to fine tuning of the signaling pathways it regulates. Thus, allosteric inhibitors or activators of Hsp90 activity may become useful tools for system biology studies.

Among the hits identified,[11] we focused our attention on the rhamnosylated 2-(4′-hydroxyphenyl)-5-propenyl-benzofuran scaffold **1** (*Eupomatenoid-2*,[12] Fig. 1). The aglyconic part is also known as *Eupomatenoid-6* (**2**, Fig. 1), a natural product extracted from the leaves of *Piper fulvescens* that has been the subject of several syntheses.[13–16] Glycodiversification[17–19] of **1** has the potential to generate a set of diverse modulators of Hsp90 activity.[9,20,21]

In this paper, we report our studies on the chemical glycosylation of *Eupomatenoid-6* (**2**).

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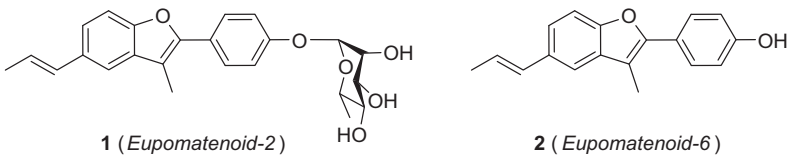


Figure 1. The rhamnosylated 2-phenyl-5-propenyl-benzofuran scaffold **1** and its aglycon **2**.

2. Results and discussion

Eupomatenoid-6 (**2**) was prepared according to a reported procedure[13] shown in Scheme 1, that starts from 2-bromo-4-chlorophenol **3** and leads to the intermediate 5-chlorobenzofuran **4**, which is transformed in **2** using a Stille coupling. Due to the diasteroemeric purity of the Stille reagent used, the target **2** was obtained as a 3:1 *E*/*Z* mixture.

For the exploration of different glycosylation strategies the 5-chlorobenzofuran **6** (Scheme 1) was chosen as a model aglycon, because it is synthetically more accessible than **2** and avoids the analysis of *E*/*Z* mixtures. Compound **6** was obtained in good yield by demethylation of **4** using sodium ethanethiolate.[13]

Glycosylation of phenols is associated with several specific problems.[22,23] First, under acidic conditions, phenols are weaker nucleophiles compared to alcohols, because the aromatic ring is electron withdrawing. Moreover, under classical Lewis acid catalyzed glycosylation conditions, C-glycosylation competes with, or often prevails over, O-glycosylation. Nonetheless, acceptable yields can usually be obtained even with not very active glycosyl donors (such as *O*-acetates) using phenols carrying electron-donating groups (e.g., *p*-methoxyphenol).[24]

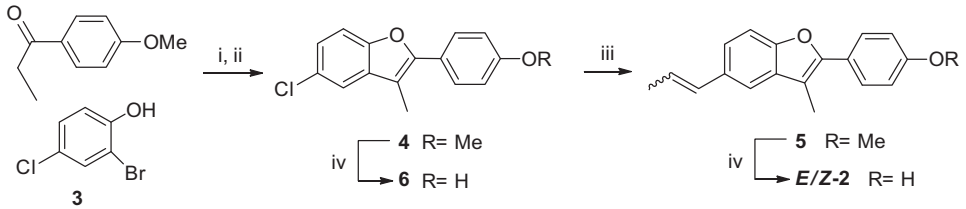
We initially tested the glycosylation of **6** with β-D-glucose penta-*O*-acetate **7** in the presence of boron trifluoride etherate[24] and with α-D-glucose tetra-*O*-acetyl-trichloroacetimidate **8**[25] in the presence of TMSOTf at −20 °C (Scheme 2). In both cases, **9** was isolated in low yields (24% and 27%) as a 6:1 (*β*/*α*) anomeric mixture and extensive decomposition of the aglycon was observed. Upon suggestion by a referee, the glycosylation reaction was also performed in the presence of boron trifluoride and TEA.[26] A slight improvement in the diastereoselectivity was indeed observed (*β*/*α*, 10:1) but yields remained disappointingly low (30% ca.).

The aglycon instability and the scarce reactivity under acidic conditions associated with poor stereoselectivity prompted us to examine glycosylation by glycosyl halide donors under basic conditions, where phenols are easily deprotonated.[23]

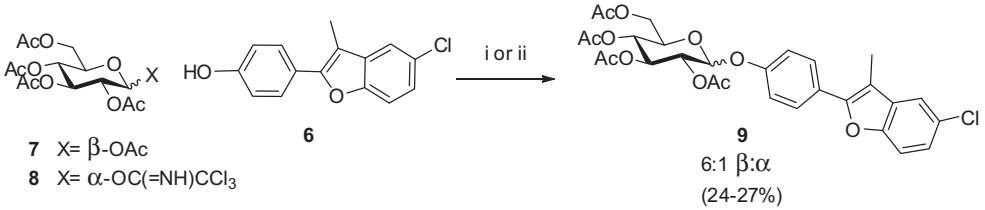
Glycosylation of model acceptor **6** with α-D-glucosyl bromide tetra-*O*-acetate donor **10** was screened under different experimental conditions (Table 1). Reaction of **10** with **6** in the presence of excess silver carbonate afforded only traces of the expected product **9** (β anomer) and the acetylated acceptor **11** was recovered as the major product (Table 1, entry 1). Reaction of the Cs salt of **6** with excess **10** in DMF at 60 °C led to recovery of unreacted starting material (Table 1, entry 2). Phase Transfer Catalysis (PTC) conditions were explored under various experimental setups. Tetrabutylammonium iodide (TBAI) was initially examined, using an excess (3 equiv) of donor in $CHCl_3$ as a solvent and aq K_2CO_3 as the base, following a reported procedure[27] (Table 1, entry 3). Under these conditions the β-glycosylation product **9** was formed in good yields (60%), but the conversion was incomplete. Similar results were obtained using the more efficient catalyst tetrabutylammonium hydrogensulfate ($TBAHSO_4$) (entry 4, 65% yield). Lowering the amount of donor (0.66 equiv, entry 5) was beneficial for the chromatographic isolation of **9**, but worsened considerably the yields of the reaction. Indeed, it was more convenient to isolate the glycosylation product after deprotection (MeONa) to yield **9a** (Table 1, entry 6, 60% yield over the two steps).

The anomeric configuration of **9** was assigned as β after deacetylation to **9a**, which showed a $J_{1,2}$ coupling constant of 7.3 Hz (DMSO-d_6, Supplementary Fig. 3).

The reaction of different glycosyl bromide donors (L-Glc, D- and L-Gal, and L-Fuc configuration) with **6** under the optimized conditions led to the desired products in modest to excellent yields (Table 2). An excess of donor or acceptor was used depending on



Scheme 1. Synthesis of *Eupomatenoid-6* (**2**) and the glycosylation model aglycon **6**. Reagents and conditions: (i) $Pd(OAc)_2$, *rac*-2-(di-*tert*-butylphosphino)-1,1′-binaphthyl (*rac*-DTBPB), NaOtBu, toluene, MW 100 °C, 1 h; (ii) TFA/DCM, rt, 45% over two steps; (iii) tri-*N*-butyl(1-propenyl)tin, $[HPtBu_3][BF_4]Pd_2dba_3$, CsF, 85%, *E*/*Z* 3:1; (iv) NaSEt, DMF, 145 °C, >95%.



Scheme 2. Glycosylation of **6** under acidic conditions. Reagents and conditions: (i) **7** (1.5 equiv), $BF_3{\cdot}Et_2O$ (3 equiv), rt. (ii) **8** (1.2 equiv), TMSOTf (0.1 equiv), −20 °C.