Communication

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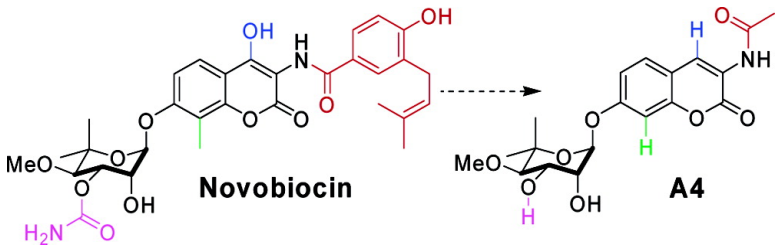


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# Hsp90 Inhibitors Identified from a Library of Novobiocin Analogues

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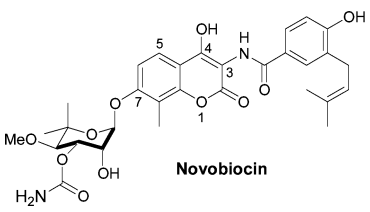
Multiple signaling pathways are upregulated or constituitively activated in malignant cells leading to (1) self-sufficiency in growth signals, (2) insensitivity to antigrowth signals, (3) evasion of apoptosis, (4) limitless replicative potential, (5) sustained angiogenesis, and (6) tissue invasion/metastasis.[1] Several anticancer agents target individual enzymes/proteins involved in the above processes, but no clinically available antitumor agent is capable of simultaneously inhibiting all six hallmarks of cancer.[1]

The 90 kDa heat shock proteins (Hsp90) are essential for refolding denatured proteins as well as for the conformational maturation of nascent polypeptides into biologically active three-dimensional structures.[2] Several Hsp90-dependent client proteins have been identified, including Raf-1, HER2, Src-family kinases, steroid hormone receptors, polo-1-kinase, death domain kinase, protein kinase B, focal adhesion kinase, telomerase, hypoxia inducible factor, and MET kinase.[2] Consequently, Hsp90 has emerged as a promising biological target for the development of cancer therapeutics because signaling nodes regulating all six hallmarks of cancer can be simultaneously disrupted by inhibition of the Hsp90 protein folding machinery.[3]

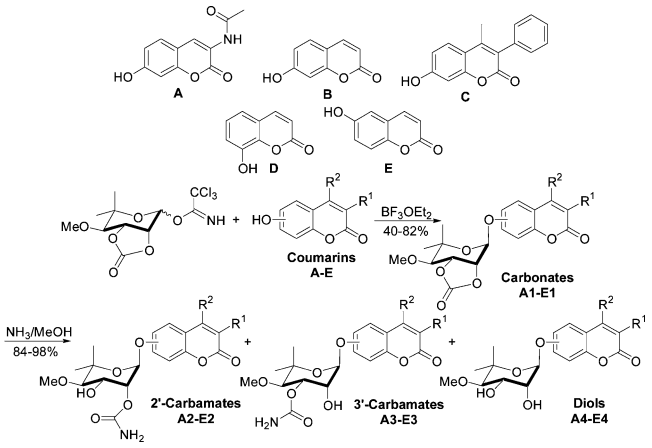
Hsp90 contains two nucleotide-binding sites; the N-terminal ATP binding site is the region to which geldanamycin (GDA) and radicicol bind[4] and the C-terminus, which was recently shown to bind novobiocin, a coumarin-containing DNA gyrase inhibitor.[5] Although other DNA gyrase inhibitors may also inhibit Hsp90, the coumarin antibiotics have so far proven the most promising of this type. Unfortunately, novobiocin's ability to cause degradation of Hsp90 clients is relatively weak (~700 $\mu$M in SKBr3 cells)[6] and requires further scientific investigation for the development of more potent compounds.




In this communication, we report the synthesis and identification of a novobiocin analogue that is substantially more active than the parent compound. These results clearly indicate that novobiocin derivatives represent a promising, unexplored class of anticancer agents that act by inhibition of the Hsp90 protein-folding machinery.

A library of novobiocin analogues was prepared that included shortening of the amide side chain and removal of the 4-hydroxy

***Scheme 1***




substituent (**A**),[7] removal of both the 4-hydroxy and amide linker (**B**), steric replacements of both the 4-hydroxy and benzamide ring (**C**), and 1,2-positional isomers of the noviosyl linkage (**D** and **E**).

These selected coumarin rings were coupled with trichloroacetimidate of noviose carbonate[8] in the presence of boron trifluoride etherate, as shown in Scheme 1.[9] The resulting cyclic carbonates (**1**) were treated with methanolic ammonia to provide 2′-carbamoyl (**2**), 3′-carbamoyl (**3**), and descarbamoyl products (**4**) in good yields.

Inhibition of Hsp90 results in the degradation of Hsp90-dependent clients via ubiquitination of the unfolded client followed by proteasome-mediated hydrolysis.[2,3] To test whether Hsp90 client proteins were degraded in the presence of these novobiocin analogues, each member of the library was incubated with SKBr3 breast cancer cells at a concentration of 100 $\mu$M. Western blot analysis of the protein lysates demonstrated that several of the compounds were capable of causing the degradation of the Hsp90-dependent client protein, phospho-AKT as represented in Figure 1. Phospho-AKT was chosen as a client protein for this assay because of previous reports indicating that phospho-AKT is a more sensitive indicator of Hsp90 inhibition than AKT.[10] Geldanamycin (GDA, 0.5 $\mu$M)[3] was used as a positive control to determine the relative ratio of phospho-AKT in the same assay.

The most active compound identified in this assay was **A4**, which contains an *N*-acetyl side chain in lieu of the benzamide, lacks the 4-hydroxyl of the coumarin moiety, and has an unmodified diol. Structure−activity relationships for these compounds suggest that attachment of the noviose moiety to the 7-position of the coumarin ring is important for biological activity (**B** vs **D** and **E**). Incorporation of the amide linker (**A**) resulted in greater inhibitory activity than the unsubstituted derivative, **B**. It is likely that the diol (**4**) mimics the ribose ring in the normal substrate (ATP) and may

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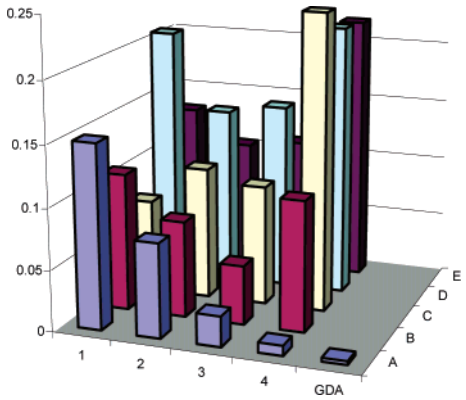


***Figure 1.*** Relative ratios of phospho-AKT by Western blot analyses. Total protein concentration of each lysate was determined, and equal amounts of protein were run in each lane of the gels. For the graphs shown in Figure 1, the O.D.'s (optical density) of the Western bands for phospho-Akt were measured, as were the O.D.'s for actin probed as controls on the same blots. To obtain the graphed values, all specific O.D.'s (for Hsp90 clients) were normalized to the respective actin O.D.

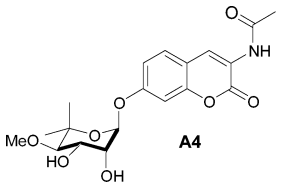


***Figure 2.*** The chemical structure of **A4**.

explain why replacement with a cyclic carbonate (**1**) or 2′-carbamate (**2**) resulted in a loss of activity.

The steroid hormone receptors are also dependent upon the Hsp90 protein folding machinery for activation and hormone binding.[2] To determine whether **A4** had similar effects on the androgen receptor, **A4** was tested in both a mutated androgen receptor-dependent prostate cancer cell line (LNCaP) and a wild-type androgen receptor prostate cancer cell line (LAPC-4). As can be seen in Figure 3, **A4** had a dramatic effect on the concentrations of the mutant androgen receptor, AKT, and Hif-1α at ∼1 $\mu$M in the LNCaP cell line. **A4** drastically reduced levels of the androgen receptor at lower concentrations in the wild-type androgen receptor prostate cancer cell line (LAPC-4). To verify that **A4** was not affecting other transcriptional or translational processes that could account for decreased protein, Hsp90 levels were determined. Under normal conditions, Hsp90 binds heat shock factor 1 (HSF1), but in the presence of Hsp90 inhibitors, this interaction is lost and HSF1 is able to induce Hsp90 expression. In this work, we showed that Hsp90 levels were significantly increased in a manner dependent upon the concentration of **A4**, consistent with similar results previously obtained by incubation with geldanamycin and radicicol. Both of these data are in contrast to actin, which is not an Hsp90 client protein and, thus, remains unaffected by Hsp90 inhibitors.[11]

In conclusion, we have prepared and evaluated a small library of novobiocin analogues, from which we have identified **A4** as a potent inhibitor of the Hsp90 protein-folding process. Further structure−activity relationships of **A4** with Hsp90 are likely to

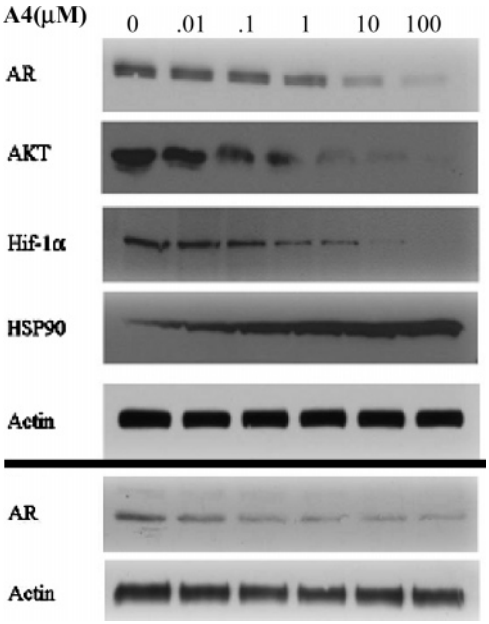


***Figure 3.*** (Top) Western blot analysis of LNCaP cells treated with **A4**. (Bottom) Western blot analysis of LAPC-4 cells incubated with **A4**. Actin was used as a control in both assays.

provide compounds with increased inhibitory activity and may ultimately provide clinically useful alternatives to GDA. Studies are underway to provide improved analogues of **A4** and to determine its activity in vivo. The results from such studies will be reported in due course.

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**Supporting Information Available:** Full experimental procedures and characterization for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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