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# Synthesis and Evaluation of Coumermycin A1 Analogues that Inhibit the Hsp90 Protein Folding Machinery

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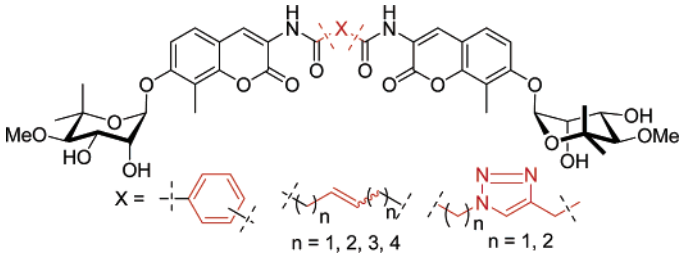
## ABSTRACT




**The coumarin antibiotics are not only potent inhibitors of DNA gyrase but also represent the most effective C-terminal inhibitors of 90 kDa heat shock proteins (Hsp90) reported thus far. In contrast to the N-terminal ATP-binding site, little is known about the Hsp90 C-terminus. In addition, very limited structure–activity relationships exist between this class of natural products and Hsp90. In this letter, the syntheses of dimeric coumarin analogues are presented along with their inhibitory values in breast cancer cell lines.**

The 90 kDa heat shock proteins (Hsp90) have quickly emerged as a promising therapeutic target for the treatment of cancer.[1] Because of their role as molecular chaperones, Hsp90 are responsible for the conformational transformation of nascent polypeptides into biologically active native structures.[2,3] Remarkably, substrates dependent upon the Hsp90 protein folding machinery are generally those that are essential to malignant progression, including transcription factors, kinases, and other proteins such as telomerase.[4]

The N-terminal ATP-binding site is the region to which the natural products geldanamycin, herbimycin A, and radicicol bind.[5] The C-terminal binding site was only recently identified,[6] and inhibitors of this motif are currently being pursued.[7] Pioneering work by the Neckers laboratory determined that the coumarin antibiotics (Figure 1) bind to the C-terminal ATP-binding site,[8] and recent collaborative studies have resulted in the identification of a simplified analogue of novobiocin that possesses increased Hsp90 inhibitory activity, A4.[7] Because the dimeric natural product, coumermycin A1 ($IC_{50} \sim 70\ \mu M$), was shown to be more effective than the monomeric species ($IC_{50} \sim 700\ \mu M$),[8] we hypothesized that dimeric variants of A4 would also exhibit increased inhibitory activity against the Hsp90 protein folding machinery. In an effort to fully investigate this group of natural product analogues, two practical approaches were undertaken. The first approach involved preparation of A4 dimers that were linked through *meta*- and *para*-phthalic acid, whereas the second approach utilized the cross-metathesis of olefins to generate a series of compounds that contained various methylene spacers in the tether.

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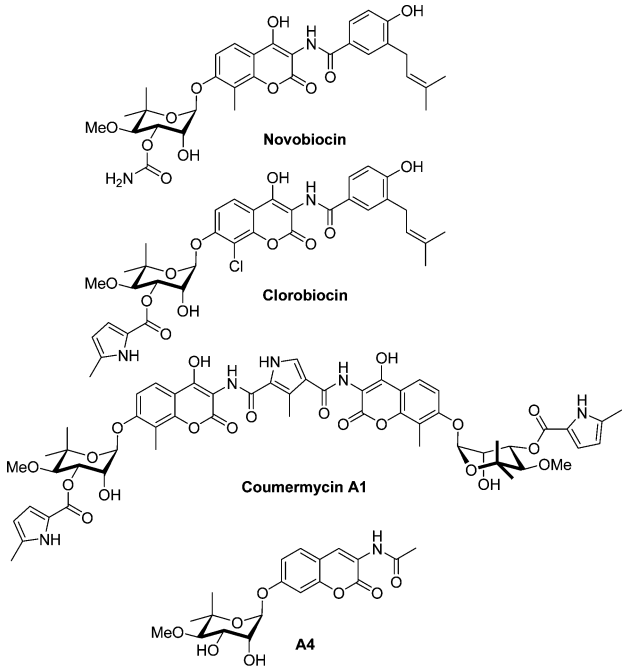
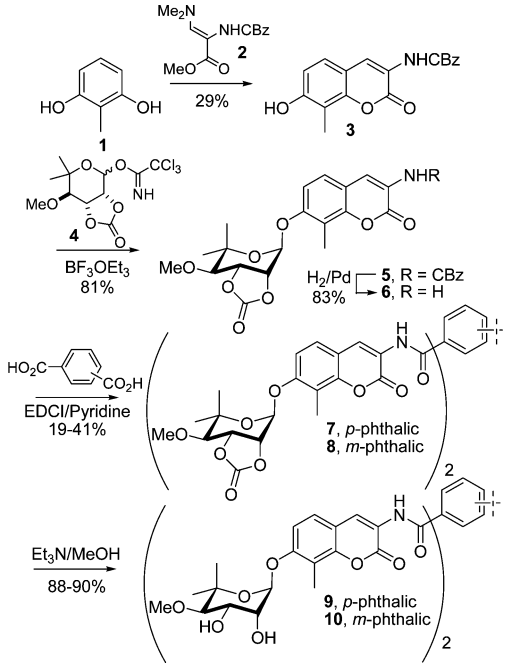



**Figure 1.** Coumarin antibiotics and A4.

**Scheme 1.** Synthesis of Phthalic Acid Dimers

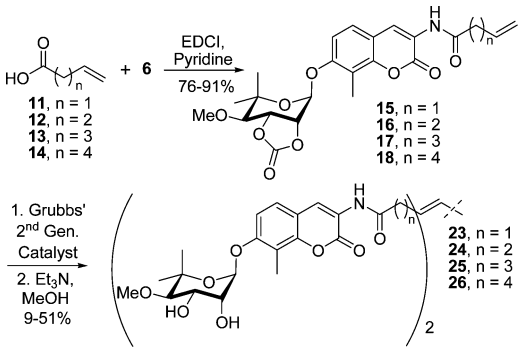



The phthalic acid derivatives were originally proposed to represent steric mimics of the pyrrole linker that is responsible for dimerization of the novobiocin monomers found in coumermycin A1. It was believed that either the para or meta derivative would be the best substitute for this moiety from an arylogous perspective. Although the ortho variants were pursued, steric congestion surrounding this construct proved detrimental as coupling was easily thwarted by the accumulation of undesired products.

Preparation of these aromatic dimers began with CBz-protected coumarin **3**, which was prepared from 2-methylresorcinol (**1**) in one step via condensation and alkylation with vinylogous carbamate **2** (Scheme 1).[9] The resulting phenol was subsequently treated with the trichloroacetimidate of noviose carbonate (**4**)[10,11] to furnish the noviosylated product, **5**. Hydrogenolysis of the CBz group furnished aminocoumarin **6**, which was coupled with *meta*- or *para*-phthalic acid to afford the dimeric products **7** or **8**, respectively. Previous results from our laboratory indicated that the noviose diol of A4 was more potent than the corresponding carbamate,[7] which is present in the natural product. Therefore, the cyclic carbonate was removed with triethylamine in methanol to provide the phthalic acid-derived dimers, **9** and **10**.

Upon construction of the aromatic-based dimeric structures, we turned our attention to a more flexible linker that can accommodate a number of conformations and perhaps enable the compounds to bind both putative ATP-binding sites in the Hsp90 homodimeric C-termini.[12,13] Preparation of these compounds was based on the cross-metathesis of individual monomeric A4 analogues. The monomers were prepared from aminocoumarin **6** as illustrated in Scheme 2. Coupling of the amine with olefinic acids **12**−**14** produced amides **15**−**18**, respectively. The resulting monomeric species underwent solvolysis to afford the corresponding diols, **15a**−**18a**, respectively. However, the carbonates were substrates for cross-metathesis when treated with Grubbs' second-generation catalyst[14] at 0.3 M for 12 h to afford

**Scheme 2.** Synthesis of Olefinic Dimers

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products **19**−**22** (not shown), which were thermodynamically derived as evidenced by the predominate trans geometry of the resulting olefins. Although the yields were not high, they were well within reported values for less complex substrates.[15] With the desired products in hand, solvolysis of the cyclic carbonates were then performed to furnish the corresponding diols, **23**−**26**.

To determine their efficacy as Hsp90 inhibitors, **9** and **10** were first evaluated in antiproliferative assays with MCF-7 and SKBr3 breast cancer cells as well as in a Her2 ELISA protocol.[16,17] To our surprise, the compounds derived from phthalic acid produced no activity, even at concentrations up to 100 $\mu$M, suggesting that this moiety is not a good replacement for the pyrrole linker found in the natural product, coumermycin A1. In contrast, both the monomers and the dimers containing the olefinic linkers proved to be active in our assays as shown in Table 1. The optimal tether length identified from these studies consisted of eight carbons, **24**.

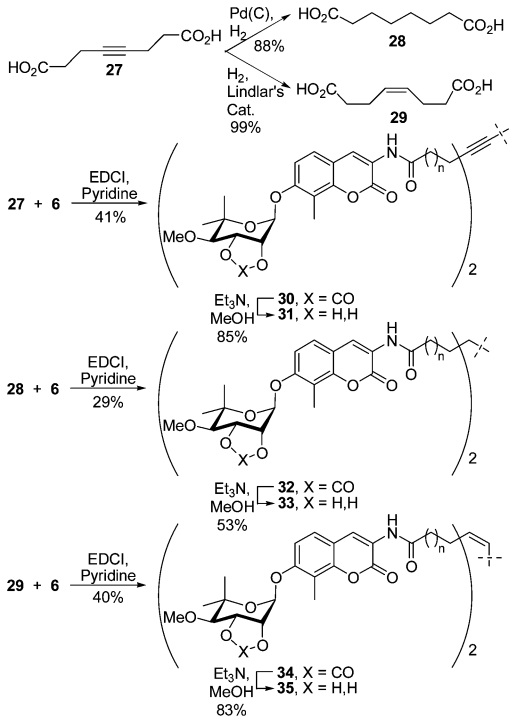
**Table 1.** Antiproliferative and Her2 Induced Degradation Activities of Coumermycin A1 Analogues Reported in $\mu$M ($n = 3$)

| entry ($IC_{50}$) | MCF-7 | SkBr3 | Her2 ELISA |
|---|---|---|---|
| **9** | >100 | >100 | >100 |
| **10** | >100 | >100 | >100 |
| **15a** | 26.6 ± 0.7 | 34.9 ± 11.0 | >100 |
| **16a** | 6.7 ± 0.5 | 5.5 ± 1.5 | 5.0 ± 0.4 |
| **17a** | 6.2 ± 0.5 | 8.4 ± 1.8 | 11.9 ± 1.9 |
| **18a** | 15.6 ± 1.5 | 28.1 ± 4.6 | 10.3 ± 2.9 |
| **23** | 53.1 ± 7.1 | >100 | 82.9 ± 4.3 |
| **24** | 3.9 ± 0.7 | 1.5 ± 0.1 | 5.6 ± 1.3 |
| **25** | 13.7 ± 3.1 | 16.7 ± 7.2 | 9.6 ± 2.4 |
| **26** | 67.4 ± 5.1 | >100 | 10.5 ± 0.3 |
| novobiocin | 352 ± 54 | 464 ± 2 | 357 ± 3.0 |
| coumermycin A1 | 5.0 ± 0.1 | 8.8 ± 0.1 | 1.6 ± 1.2 |

With the optimal chain length in hand, we wished to examine the effects of olefin geometry on inhibitory activity. We therefore pursued the synthesis of alkyne diacid **27** (Scheme 3), which represents a suitable intermediate for construction of not only the alkyne product, but also the cis and saturated derivatives.[18] Diacid **29** was prepared by Lindlar reduction, whereas **28** was furnished by hydrogenation of **27**. The resulting acids were coupled with **6** following our previously described protocol, enlisting EDCI and pyridine to afford **30**, **32**, and **34**. The cyclic carbonates were treated with methanolic triethylamine to furnish diols **31**, **33**, and **35** for biological evaluation.

Upon completion of their syntheses, compounds **31**, **33**, and **35** were evaluated for antiproliferative activity against

**Scheme 3.** Synthesis of Tether Analogues




MCF-7 breast cancer cells. The $IC_{50}$ values obtained for these compounds were 16.2, 2.7, and 23.9 $\mu$M, respectively, clearly indicating that the geometry of the tether is important for inhibitory activity (Table 2). Interestingly, the more flexible

**Table 2.** Antiproliferative and Her2 Induced Degradation Activities of Coumermycin A1 Analogues Reported in $\mu$M ($n = 3$)

| entry ($IC_{50}$) | MCF-7 | SkBr3 | Her2 ELISA |
|---|---|---|---|
| **31** | 16.2 ± 0.2 | 82.2 ± 0.7 | 95.2 ± 1.6 |
| **33** | 2.7 ± 1.0 | 1.9 ± 0.2 | 6.7 ± 1.3 |
| **35** | 23.9 ± 5.4 | 27.6 ± 2.9 | 86.9 ± 7.8 |
| **41** | 56.6 ± 5.6 | 53.7 ± 4.8 | 9.3 ± 3.6 |
| **42** | 81.0 ± 4.1 | 91.9 ± 1.4 | 85.6 ± 5.0 |

derivative **33** proved to be most active. The saturated derivative (**33**) was approximately 2-fold more active than the trans isomer, and we proposed that this hydrophobic linker may actually be solvent exposed upon binding to Hsp90. Therefore, we postulated that a triazole linker, which mimicked the trans geometry of **24**, could provide additional solubilization and hydrogen-bonding interactions and may lead to increased inhibitory activity. To this end, we prepared the related triazoles via coupling of **6** with the requisite azides and alkyne, respectively (Scheme 4). Following the procedure of Sharpless and co-workers,[19] azides **38** and **39** underwent smooth cyclization with alkyne **36** to afford the triazole

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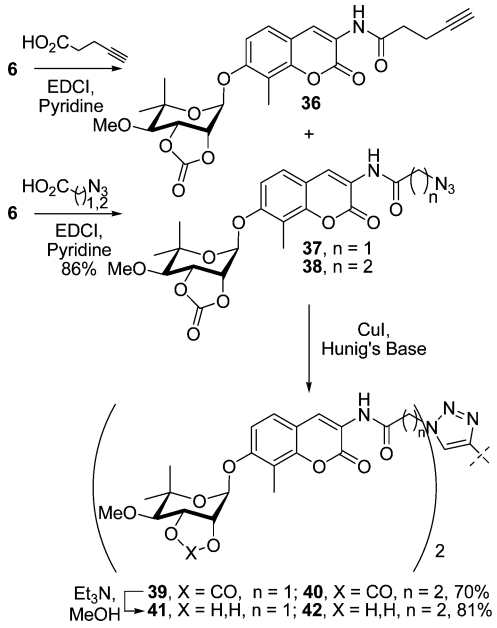
**Scheme 4.** Synthesis of Triazole Dimers




products, the carbonates of which were then solvolyzed to afford diols **41** and **42**.

Inhibitory values for **41** and **42** were obtained in MCF-7 and SKBr3 cell lines and determined to exhibit $IC_{50}$ values of $>50\ \mu M$, suggesting that the homologated pyrrole linkers were not advantageous with respect to Hsp90 inhibitory activity.

Finally, to confirm that the biological activity manifested by **33** occurred via inhibition of the Hsp90 protein folding machinery, **33** was further evaluated for its ability to induce degradation of Hsp90-dependent client proteins, Her2 and c-Raf. As shown in Figure 2, administration of **33** resulted in the degradation of Hsp90-dependent substrates in a concentration-dependent manner that links client protein degradation to the inhibition of cell growth. In addition, actin

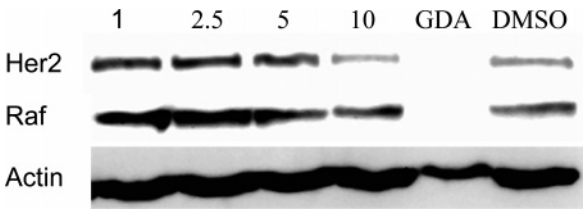



**Figure 2.** Western blot analyses of Hsp90 client protein degradation assays. Concentration of inhibitors (in $\mu M$) is denoted above each lane.

levels (control) remained unchanged, indicating that non-Hsp90-dependent proteins were not degraded in the presence of this drug.

In conclusion, we have identified the optimal tether for dimerization of the A4 scaffold and have determined that these dimeric molecules exhibit increased inhibitory activity against the Hsp90 protein folding process. In addition, we have determined that the geometry of the olefin responsible for dimerization is critical for inhibitory activity. These data suggest that the C-terminal ATP-binding regions are in close proximity to one another and that there exists a fixed distance between these binding sites. Studies are currently underway to elucidate the C-terminal ATP-binding pocket utilizing a variety of approaches, including these new drugs. The results from such studies will be reported in due course along with optimized derivatives of **33**.

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

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