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Convergent Synthesis and Discovery of a Natural Product-Inspired Paralog-Selective Hsp90 Inhibitor

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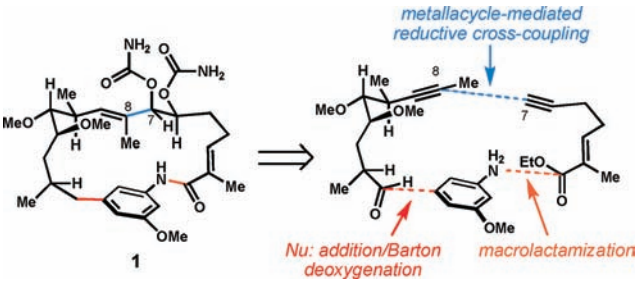
ABSTRACT



A convergent synthesis of benzoquinone ansamycin analogs is described that proceeds by a sequence of metallacycle-mediated alkyne−alkyne coupling, followed by site- and stereoselective dihydroxylation and global carbamate formation. These studies have led to (1) validation of alkyne−alkyne coupling to produce geldanamycin analogs that lack the problematic quinone, (2) the discovery that C6−C7 bis-carbamate functionality is compatible with Hsp90 inhibition, and (3) the identification of 1 as a nonquinone geldanamycin-inspired paralog-selective Hsp90 inhibitor.

Benzoquinone ansamycins are polyketide-derived natural products that display a broad range of antitumor, antibacterial, antifungal, and antiprotozoal activities (Figure 1A).[1] While efforts in chemical synthesis have targeted this natural product class for over 30 years, with the first total synthesis appearing in 1989,[2] the discovery that benzoquinone ansamycins are potent inhibitors of Hsp90 has led to a substantial increase in activity in this area with the goal of developing novel anticancer chemotherapeutic agents.[3] Hsp90 is widely held as a promising therapeutic target for cancer, as it plays a central role in controlling the post-translational conformational maturation and activation of a large number of oncogenic client proteins in a highly regulated and ATP-fueled manner (i.e., Bcr-Abl, Raf-1, HER2, Cdk4, Akt, ErbB2, and HIF-1α).[4] Additionally, a number of mutant oncoproteins require Hsp90 function, whereas their wild-type counterparts are either not dependent or only weakly dependent upon this machinery (v-SRc, EGFR, B-Raf).[5] Further enhancing the potential value of Hsp90 as a therapeutic target is the fact that it is constitutively expressed at 2- to 10-fold higher levels in tumor cells compared to their "normal" counterparts.[6] Overall, inhibition of Hsp90 results in the proteasome-mediated depletion of a number of proteins involved in cell signaling, proliferation, survival, immortalization, invasion,

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metastasis, and angiogenesis. This promising profile is further enhanced by a fundamental difference in the nature of Hsp90 in cancer cells, where it is maintained in an activated multichaperone complex that binds 100 times more tightly to Hsp90 inhibitors than Hsp90 from normal cells.[7]

The ready availability of geldanamycin from fermentation promptly placed it at the center of early studies aiming to advance a clinically relevant chemotherapeutic agent. Early studies revealed a number of problems with this natural product that included poor solubility, dose-limiting toxicity, cellular instability, and nearly equipotent activity for inhibition of GRP94—an endoplasmic reticulum paralog of the Hsp90 family whose clients include Toll-like receptors, integrins, and immunoglobulins (IgGs). While derivatives have been advanced with enhanced solubility profiles (17-AAG, IPI-504, and 17-DMAG; Figure 1B) and paralog selectivity, these semisynthetic derivatives retain the metabolic liability associated with the quinone/hydroquinone moiety central to the natural benzoquinone ansamycin skeleton.[8] Recently, related natural products that lack the problematic quinone have been discovered, synthesized, and demonstrated to also have potent Hsp90-inhibitory profiles (i.e., reblastatin and autolytimycin, Figure 1C), albeit harboring molecular features that likely impart similarly suboptimal solubility profiles.[9]

We have initiated a project to secure a robust synthesis pathway to this class of natural products to enable a search for natural product-like Hsp90 inhibitors that is unbound by constraints associated with biosynthesis, thereby providing maximum flexibility to discover superior candidates for development. Here we describe our first-generation chemical strategy, the synthesis of a focused panel of synthetic ansamycins, and the identification of a natural product-like lead as a paralog-selective Hsp90 inhibitor.

Our initial foray in to the benzoquinone ansamycins resulted in a concise (15-step longest linear) synthesis of macbecin I (Figure 2A).[10] While defining a successful chemical synthesis, this study suffered from a technical challenges that included (1) difficulties establishing the C15 stereocenter with a high degree of control, (2) the establishment of the quinone proceeded in low-moderate yield, (3) the Ti-mediated alkyne−aldehyde coupling provided a mixture of stereoisomers at C7, the minor product of which was not advanced to the natural product, and (4) the polyunsaturated C1−C7 fragment employed was quite sensitive and difficult to work with. While these issues did not

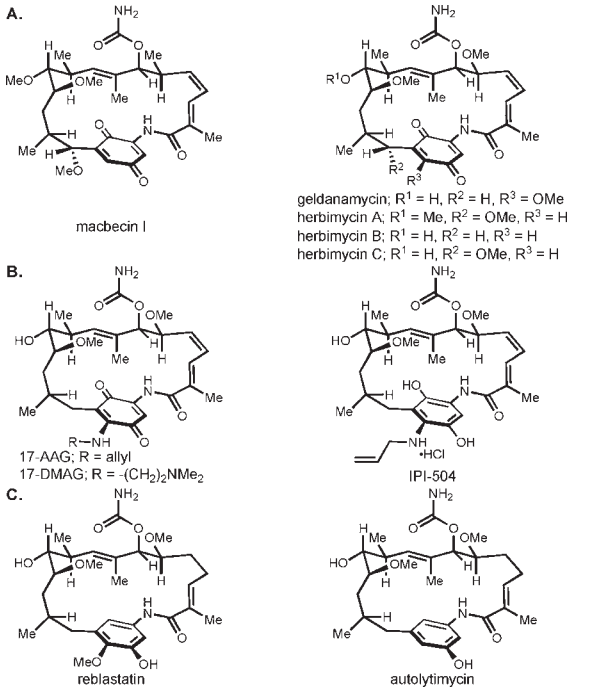

Figure 1. Introduction to benzoquinone ansamycin Hsp90 inhibitors and related natural products.

interfere with a successful synthesis of the natural product, they were perceived to be liabilities for the current pursuit.

As depicted in Figure 2B, we targeted a chemical pathway to the ansamycin skeleton that would afford derivatives that (1) lack stereochemical information at C15 (as in geldanamycin), (2) possess a saturated C4−C5 linkage (as in reblastatin and autolytimycin), (3) lack the central quinone,[11] and (4) harbor distinct C6 ether functionality.

These design criterea led to the synthesis strategy summarized in Figure 2C. Here, the C7−C8 linkage is targeted by metallacycle-mediated reductive coupling between alkynes,[12] a feature that was anticipated to resolve our previous issues with diastereoselection encountered in the alkyne−aldehyde coupling process (i.e., Figure 2A). With this basic synthesis strategy in mind, we required simple C1−C7-containing terminal alkyne coupling partners (**2**−**4**), a few suitably functionalized anilines to incorporate as the aromatic core (**5**−**7**), and sufficient quantities of the stereodefined C8−C15 alkyne-containing subunit (**8**) to enable late-stage coupling sequences for analog generation (Figure 3).[13] The rationale for selecting these coupling

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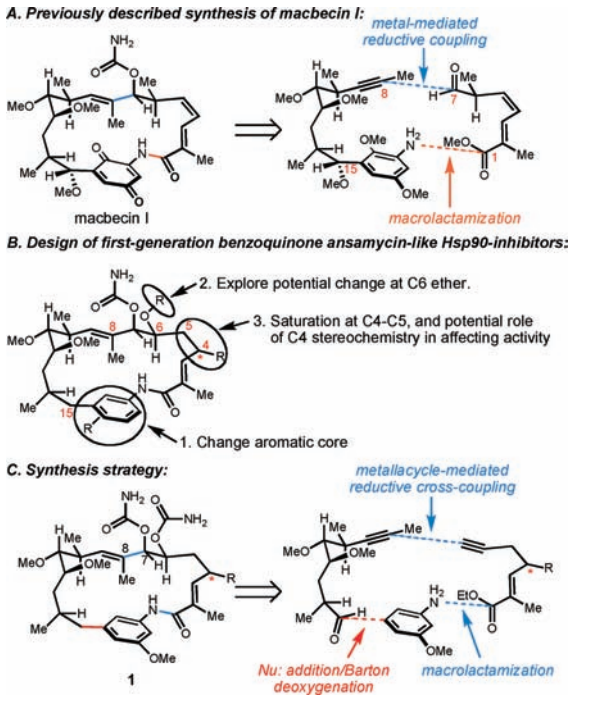

Figure 2. From a synthesis of macbecin I to a general strategy to nonbenzoquinone ansamycin derivatives.

partners was based on a few simple considerations: (1) exploring the impact of conformational restriction in the C1−C5 region (i.e., use of A-1,3 strain;[14] **3**, **4**) and (2) assessing the impact of aromatic motifs that contain H-bond acceptors (**5**, **6**), as well as providing a bias to support the *cis*-amide geometry of relevance in binding Hsp90 (**7**).

Beginning with efforts targeting the methoxy-substituted analogs derived from **5**, Li−halogen exchange (*t*-BuLi, Et_2O, −78 to 20 °C) followed by addition of aldehyde **8** led to the production of the benzylic alcohol **9** in 71% yield (dr = 1:1). Conversion to the corresponding xanthate (NaHMDS, CS_2, then MeI), followed by Barton deoxygenation (Et_3B, Bu_3SnH, PhMe, 20 °C)[15] and reductive deprotection of the aniline ($Pd(PPh_3)_4$, *N,N*-dimethylbarbituric acid) resulted

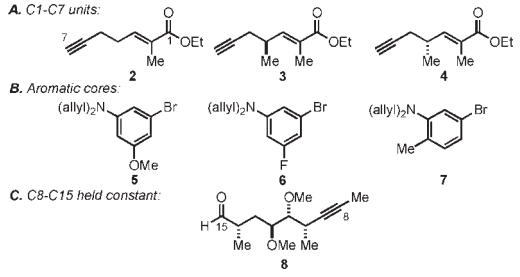

Figure 3. Coupling partners employed.

in the formation of the functionalized coupling partner **10** in 68% yield. Formation of a Ti−alkyne complex ($Ti(O\text{-}i\text{-}Pr)_4$, $c\text{-}C_5H_9MgCl$, Et_2O, −78 to −30 °C), followed by exposure to the C1−C7 terminal alkyne coupling partner **2** led to formation of the fully functionalized triene **11** in 47% yield (the remainder of the starting alkyne **10** could be isolated as its corresponding reduction product, the *Z*-alkene). Here, the C7−C8 bond was forged in concert with the establishment of each stereodefined alkene of the 1,3-diene motif (stereoselectivity ≥20:1; regioselectivity = 5:1). This demonstration of the utility of Ti-mediated reductive cross-coupling of alkynes is significant, as bimolecular C−C bond formation between the preformed Ti−alkyne complex and terminal alkyne proceeds faster than reaction with the α,β-unsaturated ester group in **2**, an electron-deficient functionality of potential sensitivity in reactions with preformed organometallic intermediates.

Moving on, saponification of the ethyl ester (LiOH, THF, MeOH, H_2O) and macrolactamization (BOPCl, $i\text{-}Pr_2NEt$, PhMe, 80 °C) delivered the macrocyclic triene **12** in 59% yield. Site and stereoselective dihydroxylation was then accomplished under the conditions of Sharpless[16] and generated the stereodefined diol **13** in 60% yield as a single isomer. Finally, formation of the bis-carbamate proceeded by sequential treatment with trichloroacetyl isocyanate and $i\text{-}Pr_2NEt$/MeOH to generate the fully functionalized analog **1** in nine steps from the coupling partners.[17] While this final functionalization reaction reproducibly proceeded in poor yield,[18] the process was sufficient to generate material suitable for biochemical evaluation. Efforts to optimize this final step will be addressed in future studies.

Using the general aspects of the synthesis pathway depicted in Figure 4, a collection of benzoquinone ansamycin derivatives were prepared (Figure 5A). These derivatives vary with respect to (1) the nature of the aromatic core, (2) the substitution and stereochemistry at C4, and (3) the nature of the functionality at C6−C7. It is well-known that the C7-carbamate of the benzoquinone ansamycins plays an important role in binding to Hsp90, and as such, we targeted derivatives that uniformly retain this functionality. Surprisingly, during the course of our studies, we observed that the final hydrolysis reaction for generating the carbamate at C7 (of the resulting *N*-trichloroacetyl carbamate) was

(13) The C8−C15 subunit was available using our previously described synthesis strategy, while the other subunits were prepared by well-established methods. See the Supporting Information for details.

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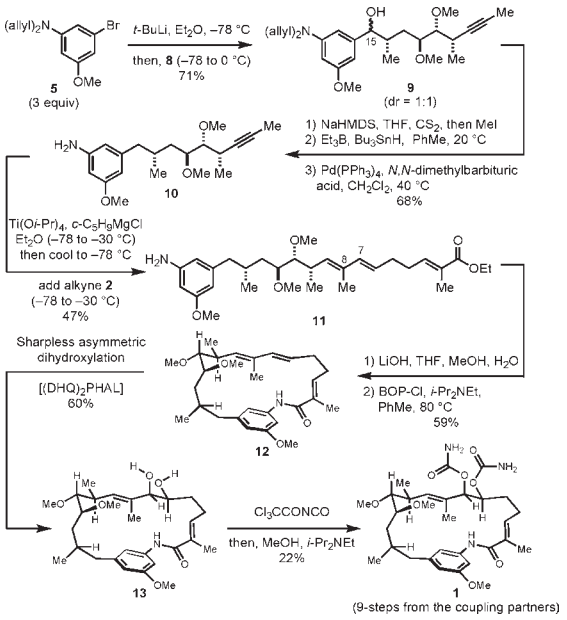
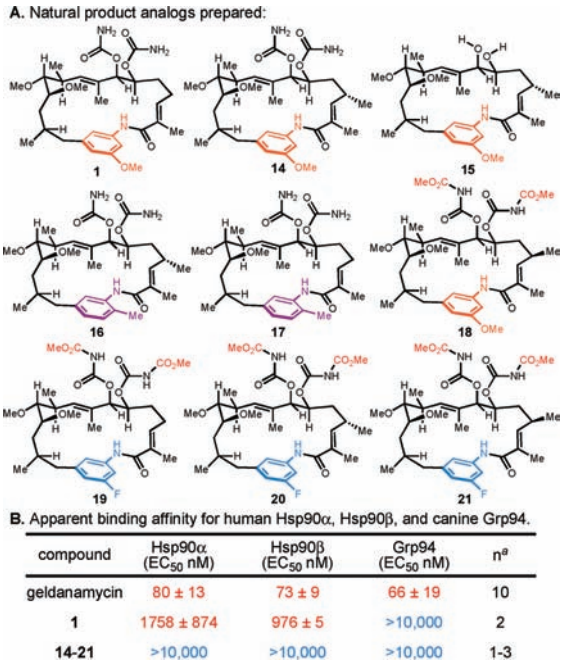


Figure 4. Synthesis pathway employed for ansamycin analogs.

unpredictable, generating in some cases the corresponding imides (**18**–**21**).[19]

To evaluate the Hsp90 inhibitory potential of the natural product-like derivatives prepared, we utilized fluorescence polarization (FP) competition binding assays for Hsp90α, Hsp90β, and GRP94, the endoplasmic reticulum paralog of Hsp90.[20] Consistent with previous reports, geldanamycin was found to be a potent binder to all three proteins with an apparent EC_{50} near 70 nM. Perhaps not surprisingly, all imide-containing derivatives (**18**–**21**) lacked affinity for any of the biological targets explored ($EC_{50} \geq 10\ \mu M$). Interestingly, for the remaining series (**1**, **14**–**17**), only compound **1** showed substantial affinity for Hsp90α and Hsp90β with EC_{50}'s of 1.7 μM and 980 nM, respectively (Figure 5B), yet having no affinity for GPR94 up to 10 μM. These results reveal that C6 carbamate functionality is compatible with Hsp90 inhibition and offers a means to accomplish paralog selectivity between Hsp90 and GRP94. Further, the lack of affinity associated with **14**–**17** demonstrates the sensitive nature of this compound class with respect to changes in substitution at C4 and the aromatic nucleus.



compound	Hsp90α (EC_{50} nM)	Hsp90β (EC_{50} nM)	Grp94 (EC_{50} nM)	n[a]
geldanamycin	80 ± 13	73 ± 9	66 ± 19	10
1	1758 ± 874	976 ± 5	>10,000	2
14-21	>10,000	>10,000	>10,000	1-3

Figure 5. Benzoquinone ansamycin analogs prepared. (a) EC_{50} values are from the average of 10 experiments for geldanamycin (each read in duplicate), two experiments ± standard deviation for **1** (each read in triplicate), and three experiments for **14**, **17**, and **19** (each read in triplicate).

Overall, we report the establishment of a chemical synthesis pathway to unnatural derivatives of benzoquinone ansamycins that proceeds by metallacycle-mediated alkyne–alkyne coupling. This synthesis pathway was employed to generate a preliminary panel of natural product-like analogs and led to the discovery of a paralog-selective Hsp90 inhibitor (**1**). While affinities of **1** to Hsp90 are still more than 1 order of magnitude lower than geldanamycin, the selectivity profile is unique, and the molecular skeleton lacks the problematic quinone central to geldanamycin and clinically relevant analogs. These discoveries solidify the relevance of pursuing Hsp90 inhbitors based on the C6,C7-bis carbamate functionality resident in **1**, and establish the significance of alkyne–alkyne coupling for the synthesis of related natural product-like analogs. We look forward to future developments that build on these intitial findings.

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Supporting Information Available. Experimental procedures and tabulated spectroscopic data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

(19) These compounds are most likely generated via the haloform-like expulsion of CCl_3^- during the collapse of the tetrahedral intermediate during final methanolysis. We suspect that this anomalous reaction pathway will be supressed by conducting the hydrolysis under aqueous conditions. This was not pursued in the current study but will be addressed in future work.

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