Optimizing Natural Products by Biosynthetic Engineering: Discovery of Nonquinone Hsp90 Inhibitors†

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Abstract: A biosynthetic medicinal chemistry approach was applied to the optimization of the natural product Hsp90 inhibitor macbecin. By genetic engineering, mutants have been created to produce novel macbecin analogues including a nonquinone compound (**5**) that has significantly improved binding affinity to Hsp90 (K_d 3 nM vs 240 nM for macbecin) and reduced toxicity (MTD ≥ 250 mg/kg). Structural flexibility may contribute to the preorganization of **5** to exist in solution in the Hsp90-bound conformation.

Heat-shock protein 90 (Hsp90)[a] is an ATP-dependent molecular chaperone required for the stability and maturation of numerous client proteins with a broad range of function[1] and plays an additional key role in stress response and protection of the cell against the effects of mutation.[2] Many Hsp90 client proteins are overexpressed in cancer, often in mutated forms, and are responsible for unrestricted cancer cell proliferation and survival.[3] Inhibition of Hsp90 causes destabilization and degradation of these oncogenic client proteins providing an attack on the unrestricted proliferation and cell survival characteristic of cancer cell growth. This leads to cell growth inhibition and apopotosis.[4] Hsp90 is considered a major therapeutic target for anticancer drug development because it offers the possibility of disrupting multiple oncogenic proteins simultaneously.

Although too toxic to be developed as an anticancer drug,[5] the natural product Hsp90 inhibitor geldanamycin has served as a prolific lead for semisynthetic optimization, resulting in three candidate drugs in clinical trials and a further number of derivatives in preclinical research. 17-Allylamino-17-demethoxygeldanamycin (17-AAG) (Figure 1, **2**)[6] has reduced hepatotoxicity compared to geldanamycin and is the most advanced Hsp90 inhibitor in clinical development (Phase II/III) but suffers from poor solubility.[7] With an ionizable basic amino side chain, 17-(2-dimethylamino)ethylamino-17-demethoxygeldanamycin (17-DMAG) (Figure 1, **3**)[8] is much more water-soluble than 17-AAG, although it exhibits greater

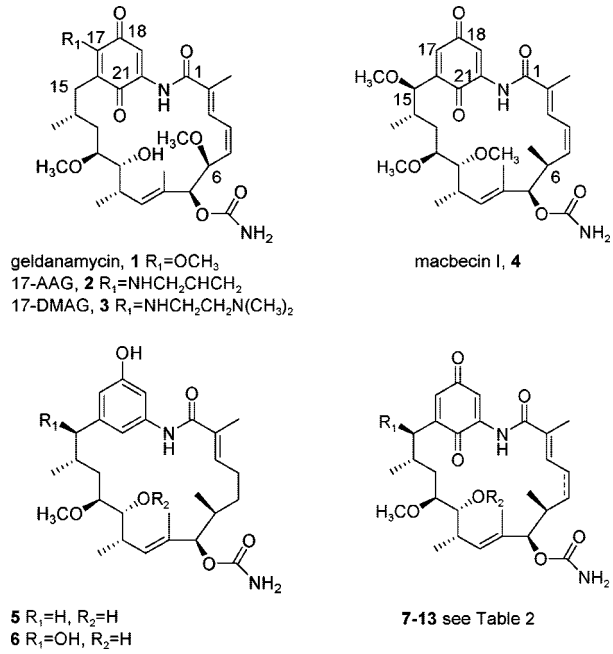

Figure 1. Chemical structures of ansamycin-class Hsp90 inhibitors.

toxicity. A reduced version of **2**, hydroquinone-17-AAG (or IPI-504), forms water-soluble salts and is also under clinical development.[9]

However, these first-generation Hsp90 inhibitors all share a common unfavorable structural feature, the benzoquinone toxicophore which undergoes redox cycling involving a NAD(P)H/quinone oxidoreductase (NQO1) catalyzed 2-electron reduction of quinone to dihydroquinone, leading to superoxide radical generation, and has been suggested as a component of their cytotoxicity.[10,11] In fact, the activity of benzoquinone ansamycins is to some degree dependent upon the expression level of NQO1. Reduction in NQO1 expression levels thus represents a mechanism of acquired resistance against these compounds.[12] 17-AAG and analogues have been shown to undergo conjugation with glutathione, via Michael attack at C17, leading to cellular depletion.[13] It is suggested that this conjugation with sulfur-containing nucleophiles may contribute to the toxicity of these quinone-containing compounds.[13] The quinone moiety of ansamycins is thus a key target feature for lead optimization. Several semisynthetic approaches have been utilized to stabilize the quinone moiety, e.g., by forming 18-oximes.[14] More radically, de novo lead identification and optimization of small synthetic molecules have been pursued to obtain Hsp90 inhibitor chemotypes that are devoid of the quinone moiety.[15]

We describe here an alternative biosynthetic approach for the optimization of ansamycin-class Hsp90 inhibitor leads represented by macbecin (Figure 1, **4**).[16] The strategy is based on our understanding of the quinone biosynthetic pathway for ansamycin polyketides, i.e., hydroxylation of a phenol precursor followed by autoxidation by molecular oxygen. Through genetic inactivation of the monooxygenase responsible, formation of the quinone can be inhibited.

The macbecin biosynthetic gene cluster was identified in *Actinosynnema pretiosum* subsp. *pretiosum* ATCC 31280 and its sequence obtained using standard methods (Accession number EU827593). The sequenced region covers 100 kbp, and 23 open reading frames were identified constituting the macbecin biosynthetic gene cluster (Figure 2 and Table S1, Supporting Information).

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† PDB code of crystal structure of **5** bound to Hsp90: 2VW5. Accession number for macbecin biosynthesis cluster: EU827593.

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[a] Abbreviations: Hsp90, heat shock protein 90; ITC, isothermal titration calorimetry; TMD, targeted molecular dynamics; NQO1, NAD(P)H:quinone oxidoreductase 1.

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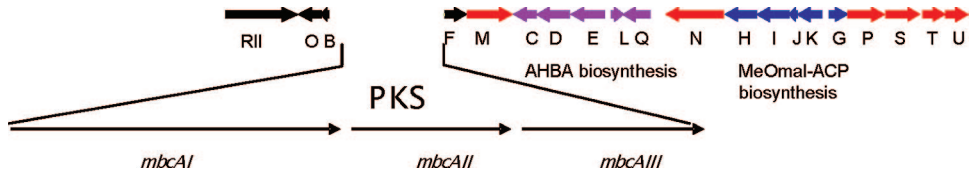

Figure 2. Biosynthetic cluster for macbecin (mbc). Genes responsible for the biosynthesis of premacbecin are shown in black. Within the cluster are genes for the biosynthesis of the aminohydroxybenzoic acid starter unit (purple) for the methoxymalonyl-ACP extender unit (blue) and for the post PKS modifications (red).

Table 1. Analogues Produced by Engineering the Macbecin Biosynthetic Pathway

gene target(s)	mutant type	complementation	product(s)
mbcM	deletion	none	**5**, **6**
mbcT	integrant	none	**7**, **8**
mbcU	integrant	none	**7**, **8**, **9**, **10**
mbcS	integrant	none	**8**, **10**
mbcT and *U*	deletion	none	**7**
mbcT and *U*	deletion	*mbcT*	**7**, **9**
mbcT and *U*	deletion	*mbcU*	**7**, **11**
mbcP, *S*, *T*, and *U*	deletion	none	**12**
mbcP	integrant	none	**12**
mbcP, *S*, *T*, and *U*	deletion	*mbcS*, *T*, and *U*	**12**, **13**

Interestingly, the macbecin cluster appears to be complete in that every gene anticipated to be required for provision of the AHBA starter unit and the unusual methoxymalonyl-CoA extender unit to each post-PKS step is present (Figure 2). This is not the case for either of the sequenced biosynthetic gene clusters for geldanamycin or herbimycin.[17] By comparison with the biosynthetic cluster of geldanamycin,[18] it was apparent that the GdmM homologue (MbcM) is responsible for the introduction of oxygen at C21. These enzymes share high homology with flavin dependent monooxygenases, consistent with this role. To suggest that GdmM is involved in formation of the benzoquinone by effecting oxidation at C21 is in contrast to the published hypothesis that it is likely to be involved in oxidation at C17 of geldanamycin but is consistent with the experimental data of that report.[17] Molecular genetic manipulation of the producing organism to delete *mbcM* yielded the strain *A. pretiosum* Δ*mbcM* (Biot-3970). Analysis of Biot-3970 fermentation broth using LCMS identified two nonquinone macbecin analogues that were isolated and characterized by NMR as **5** and **6** (Figure 1). No other macbecin analogues were observed. **5** and **6** arise as a result of polyketide synthesis to generate the first enzyme free intermediate, followed by carbamoylation of the C7-oxygen. The remainder of the post-PKS processing steps have either not taken place, or, in the case of **6**, a single hydroxylation has occurred at C15. Thus, in addition to disruption of C21-oxidation, introduction of the 4,5-olefin and *O*-methylation at C11 and C15 (when 15-hydroxy is present), all failed to occur. These data suggest that C7-*O*-carbamoylation is an early step (possibly the first) of post-PKS biosynthesis and that C21-oxidation may be the second step, leading to the accumulation of shunt metabolites that are not recognized for processing by the remaining post-PKS tailoring enzymes.

A series of mutant strains containing different combinations of post-PKS genes were generated using standard methods (Table 1, Tables S2 and S3 of Supporting Information). The predicted activity of each protein along with the macbecin analogue(s) (Figure 1, Table 2) produced from each strain defined or confirmed the gene function (Table S1, Supporting Information) and allowed us to suggest a preferred order of biosynthetic steps: C7-*O*-carbamoylation, C21-oxidation, C4,5-desaturation, C15-hydroxylation, and C11/C15-*O*-methylation.

Table 2. Structures and IC_{50} Values of Novel Macbecin Analogues

compd	C4−C5	R_1 at C15	R_2 at C11	mIC_{50}[a] (μM)
macbecin, **4**	double bond	OMe	Me	0.02
5	dihydro	H	H	0.02
6	dihydro	OH	H	3.79
7	double bond	OH	H	5.1
8	double bond	H	Me	0.16
9	double bond	OH	Me	0.19
10	double bond	H	H	0.09
12	dihydro	H	H	0.20

[a] mIC_{50} = mean IC_{50}s against 6 cancer cell lines: CNXF-498NL, HT29, LXF-1121 L, MCF7, MEXF-394NL, DU145.

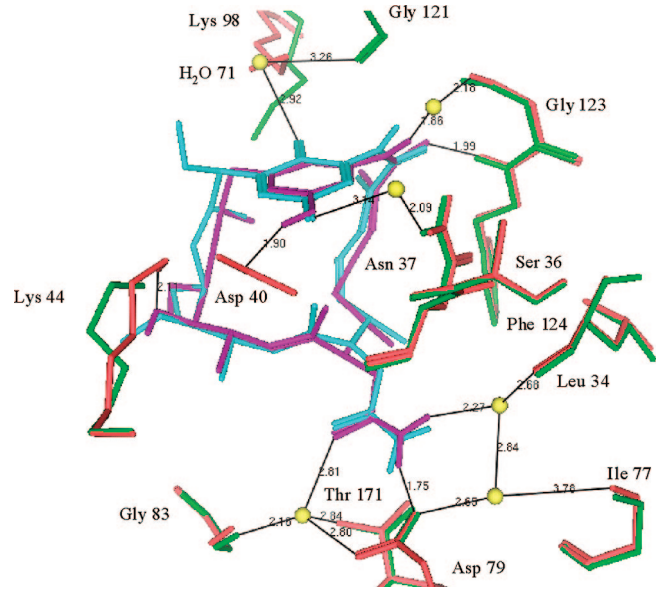

Figure 3. Superimposition of X-ray crystal structures of Hsp90-bound **5** and macbecin, **4**. Some key receptor residues involved in binding **5** are shown in red, while the relevant residues for the cocrystal structure with **4** are shown in green. Macbecin, **4**, is shown in cyan, and **5** is shown in purple. H_2Os are depicted in yellow. Other H_2Os and hydrogen-bonding interactions have been omitted for clarity.

All compounds, except **11** and **13**,[19] were isolated and structurally characterized (see Supporting Information) and submitted for inhibitory screening against a panel of six cancer cell lines (Table 2). The 15-hydroxy group appears unfavorable for cellular activity both in the nonquinone and quinone-containing compounds, i.e., **6** is much less active than **5**, and **7** is much less active than **10**, although **8** and **9** are similarly active. We then determined the binding of **5** and **6** to yeast Hsp90 protein by isothermal titration calorimetry (ITC). Both compounds showed equally high affinity to the target protein (K_d of 3 nM for **5** and 6 nM for **6** Figure S2, Supporting Information), leading us to propose that the 15-hydroxy group has an effect on cell membrane permeability and/or efflux. To produce **5** as a single fermentation product, Biot-3970 was further engineered to remove the operon containing the four genes *mbcP*, *S*, *T*, and *U*, which govern the remaining biosynthetic steps.

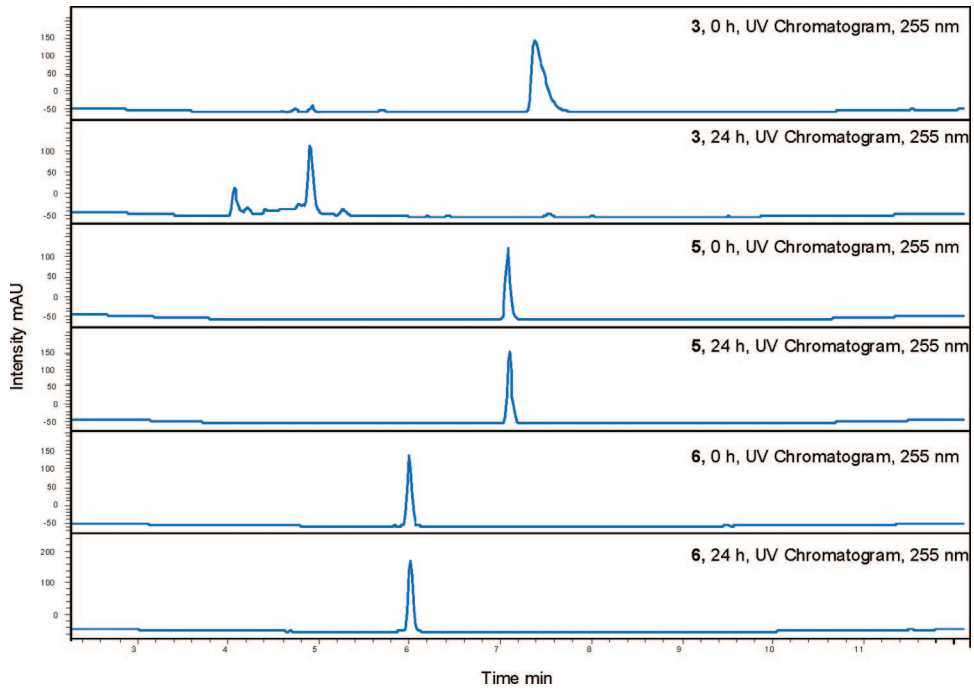

Figure 4. Conjugation of ansamycin polyketides with glutathione in PBS. 17-DMAG, **3** undergoes rapid conjugation and is completely degraded within 24 h. **5** and **6** undergo no reaction and are stable.

Cultivation of the resulting strain Biot-3982 led to the exclusive production of **5** at ≥200 mg/L.

The new nonquinone ansamycin **5** stands out as a potent Hsp90 inhibitor with almost 100-fold higher binding affinity than the natural product lead macbecin (K_d 3 nM vs 240 nM).[16] Additionally, **5** has significantly enhanced solubility compared to **4** (Table S5, Supporting Information). Examination of the X-ray crystal structure of **5** in complex with Hsp90 (PDB code 2VW5) revealed that, like macbecin, **5** binds to the N-terminal ATP binding site adopting a "folded" C-conformation with the phenol moiety located at the entrance of the pocket in place of the benzoquinone moiety of **4** and that the carbamate group is involved in a hydrogen-bonding network at the base of the active site. The most significant difference is seen for the position of Lys98 (toward the top of Figure 3), which appears to be driven by the displacement of a water (H_2O-71 in the **4**-Hsp90 structure PDB code 2VLS),[16] which allows Lys98 to more closely approach the aromatic ring of **5** (Figure 3). The complete list of interactions observed between **5** and Hsp90 is given in Table S6, Supporting Information. By comparison of the overlaid Hsp90 cocrystal structures of **4** and **5** (Figure 3), it can be seen that the presence of a phenolic moiety in **5** is advantageous to binding because the 18-phenol can interact directly with Asp40, while the corresponding 18-carbonyl group of **4** is likely to cause an unfavorable dipole interaction with Asp40, weakening its binding interaction. Additionally, conversion of the 11-methoxy to 11-hydroxy results in a stronger hydrogen-bond to Lys44 for **5** as observed in the shorter hydrogen bond length (2.1 Å) than in the **4** structure (2.3 Å).

A further important structural contributor to the improved Hsp90 binding affinity of **5** is saturation of the 4,5-olefin (formed biosynthetically by action of MbcP). Similarly to other quinone ansamycins, macbecin undergoes an energetically unfavorable conformational change in order to adopt the folded bound C-conformation with a *cis*-amide from its free solution state containing a *trans*-amide.[16] This is due in part to high structural rigidity derived from the conjugated system extending from the quinone ring to the 4,5-olefin. Saturation of the 4,5-olefin contributes to a higher population of the folded bound conformation in solution and thus has a potentially positive effect on binding affinity to Hsp90. Examination of the NOESY spectra of **5** provides correlations consistent with a folded C-conformation in solution (see Supporting Information, Figure S3), which is similar to the active forms of both geldanamycin and macbecin bound to Hsp90 (PDB code 1YET and 2VLS, respectively). This is further consistent with the observation that reblastatin, which has a similar ansamycin ring structure to **5**, i.e., no quinone or 4,5-olefin, exists in a free conformation that is nearly identical to the Hsp90 bound conformation of geldanamycin and 17-AAG.[20]

Removal of the quinone moiety improved the MTD of compound **5** compared with the parent, macbecin without compromising its potency (**5** still shows comparable cellular potency to the parent macbecin (Table 2)). We link this effect directly to decreased off-target cytotoxicity compared with quinone-containing Hsp90 inhibitors. The structures of the nonquinone Hsp90 inhibitors **5** and **6** do not allow redox cycling and do not undergo conjugation with nucleophiles. After 24 h incubation with glutathione, no adducts were observed with **5**, whereas 17-DMAG was completely converted to multiple adducts (confirmed by LCMS) as reported previously (Figure 4).[13] As a consequence of this reduction in off-target chemical reactivity, the compound is much better tolerated than quinone-containing Hsp90 inhibitors. Mice dosed ip at 250 mg/kg/day for five days with **5** showed no apparent toxicity, whereas previous studies with macbecin dosed at 10 or 20 mg/kg have shown toxicity, including unscheduled deaths.[16,21] The maximum tolerated treatment dose of 17-AAG under the same dosing regimen was 50 mg/kg. In contrast to quinone-containing Hsp90 inhibitors, the cancer cell inhibitory activities of **5** were independent of the expression levels of NQO1 (Figure S4, Supporting Information). When dosed at 85 mg/kg, i.e., $^1/_3$ of the MTD, in mice bearing a MAXF 401 human mammary carcinoma xenograft, no toxicity was apparent, and compound **5** showed equivalent in vivo antitumor efficacy (minimum $T/C = 45.3\%$, recorded on day 35) to that of 17-AAG at its maximum tolerated dose of 50 mg/kg (Figure S5, Supporting Information). Taken together these data demonstrate an improvement of therapeutic index for **5**.

In conclusion, we have demonstrated that biosynthetic engineering methodologies can be applied rationally to optimize a structurally complex natural product lead, specifically here the ansaymcin polyketide Hsp90 inhibitor macbecin. Modification to target the undesired quinone was achieved by specific gene inactivation to yield the nonquinone ansamycin Hsp90 inhibitor **5**. The absence of additional functions around the ansa ring, particularly saturation of the 4,5-olefin, affords **5** greater flexibility to readily adopt the Hsp90 bound conformation. We believe that the X-ray crystallographic studies of Hsp90-bound **5** and NMR studies of **5** in solution, along with reported structural studies regarding the related natural product reblastatin,[20] clearly support this point.

Additionally, **5** exhibits significantly enhanced binding affinity for Hsp90 compared to 17-AAG and macbecin (as measured by ITC). 17-AAG has been shown to exhibit time-dependent slow-tight binding,[22,23] with full levels of cellular inhibition taking up to 24 h to achieve.[22] We believe this time-dependent binding of 17-AAG is explained, at least in part, by the kinetics of conformational reorganization from the open form, which has been shown to involve two high-energy transitions for the related 17-DMAG,[24] to that of the folded C-conformation bound structure. We thus rationalize our accumulated data as indicating fast-tight binding of **5** to Hsp90, which may be attributable to population of the folded C-conformation structure in solution.

A further benefit of using biosynthetic engineering to optimize macbecin, as compared with the semisynthetic optimization of geldanamycin, lies in the fact that increased Hsp90 inhibitory potency and the reduced off-target toxicity have been achieved at no expense to molecular weight, which is contrary to most semisynthetic approaches. In fact, the new nonquinone Hsp90 inhibitor **5** has a molecular weight of 502 and a binding efficiency index (BEI)[25] of 16.9 compared to the lead molecule macbecin (MW 558) which has a BEI 11.9. Rational biosynthetic engineering may be a general methodology applicable for the optimization of structurally complex natural product leads.

Supporting Information Available: Experimental details and data. This material is available free of charge via the Internet at http://pubs.acs.org.

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References

(1) Pearl, L. H.; Prodromou, C. Structure and mechanism of the Hsp90 molecular chaperone machinery. *Annu. Rev. Biochem.* **2006**, *75*, 271–294.
(2) Bagatell, R.; Whitesell, L. Altered Hsp90 function in cancer: a unique therapeutic opportunity. *Mol. Cancer Ther.* **2004**, *3*, 1021–1030.
(3) Hanahan, D.; Weinberg, R. A. The hallmark traits of cancer. *Cell* **2000**, *100*, 57–70.
(4) Maloney, A.; Workman, P. Hsp90 as a new therapeutic target for cancer therapy: the story unfolds. *Expert Opin. Biol. Ther.* **2002**, *2*, 3–24.
(5) Supko, J. G.; Hickman, R. L.; Grever, M. R.; Malspeis, L. Preclinical pharmacologic evaluation of geldanamycin as an antitumor agent. *Cancer Chemother. Pharmacol.* **1995**, *36*, 305–315.
(6) Schnur, R. C.; Corman, M. L.; Gallaschun, R. J.; Cooper, B. A.; Dee, M. F.; Doty, J. L.; Muzzi, M. L.; Moyer, J. D.; DiOrio, C. I.; Barbacci, E. G.; Miller, P. E.; O'Brien, A. T.; Morin, M. J.; Foster, B. A.; Pollack, V. A.; Savage, D. M.; Sloan, D. E.; Pustilnik, L. R.; Moyer, M. P. Inhibition of the oncogene product p185erbB-2 in vitro and in vivo by geldanamycin and dihydrogeldanamycin derivatives. *J. Med. Chem.* **1995**, *38*, 3806–3812.
(7) Sausville, E. A.; Tomaszewski, J. E.; Ivy, P. Clinical development of 17-allylamino-17-demethoxygeldanamycin. *Curr. Cancer Drug Targets* **2003**, *3*, 377–383.
(8) Tian, Z.-Q.; Liu, Y.; Zhang, D.; Wang, Z.; Dong, S. D.; Carreras, C. W.; Zhou, Y.; Rastelli, G.; Santi, D. V.; Myles, D. C. Synthesis and biological activities of novel 17-aminogeldanamycin derivatives. *Bioorg. Med. Chem.* **2004**, *12*, 5317–5329.
(9) Ge, J.; Normant, E.; Porter, J. R.; Porter, J. R.; Ali, J. A.; Dembski, M. S.; Gao, Y.; Georges, A. T.; Grenier, L.; Pak, R. H.; Patterson, J.; Sydor, J. R.; Tibbitts, T. T.; Tong, J. K.; Adams, J.; Palombella, V. J. Design, synthesis, and biological evaluation of hydroquinone derivatives of 17-amino-17-demethoxygeldanamycin as potent, water-soluble inhibitors of Hsp90. *J. Med. Chem.* **2006**, *49*, 4606–4615.
(10) Kelland, L. R.; Sharp, S. Y.; Rogers, P. M.; Myers, T. G.; Workman, P. DT-diaphorase expression and tumor cell sensitivity to 17-allylamino-17-demethoxygeldanamycin, an inhibitor of heat shock protein 90. *J. Natl. Cancer Inst.* **1999**, *91*, 1940–1949.
(11) Dikalov, S.; Landmesser, U.; Harrison, D. G. Geldanamycin leads to superoxide formation by enzymatic and non-enzymatic redox cycling. Implications for studies of Hsp90 and endothelial cell nitric-oxide synthase. *J. Biol. Chem.* **2002**, *277*, 25480–25485.
(12) Gaspar, N.; Sharp, S. Y.; Pacey, S.; Jones, C.; Walton, M.; Vassal, G.; Pearson, A.; Workman, P. Acquired resistance to HSP90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG) in adult and pediatric glioblastoma cell lines. AACR2008, San Diego Convention Center, San Diego, CA, 2008; Abstract 2461.
(13) Cysyk, R. L.; Parker, R. J., Jr.; Steeg, P. S.; Hartman, N. R.; Strong, J. M. Reaction of geldanamycin and C17-substituted analogues with glutathione: product identifications and pharmacological implications. *Chem. Res. Toxicol.* **2006**, *19*, 376–381.
(14) Tao, C.; Han, H.; Sun, X.; Hwang, L.; Everett, N.; Gennity, I.; Westlake, K.; Wang, Q.; Ci, S.; Desai, N. Preparation and SAR of novel oxime derivatives of geldanamycin. AACR2008, San Diego Convention Center, San Diego, CA, 2008; Abstract 5642.
(15) (a) Chandarlapaty, S.; Sawai, A.; Ye, Q.; Scott, A.; Silinski, M.; Huang, K.; Fadden, P.; Partdrige, J.; Hall, S.; Steed, P.; Norton, L.; Rosen, N.; Solit, D. B. SNX2112, a synthetic heat shock protein 90 inhibitor, has potent antitumor activity against HER kinase-dependent cancers. *Clin. Cancer Res.* **2008**, *14*, 240–248. (b) Rice, J.; Fadden, P.; Huang, K.; Barabasz, A.; Foley, B.; Veal, J.; Steed, P.; Hall, S. Biological effects of the novel small molecule Hsp90 inhibitor SNX-2112 as a single agent and in combination with erlotinib in non-small cell lung cancer models. AACR2008, San Diego Convention Center, San Diego, CA, 2008; Abstract 5629.
(16) Martin, C. J.; Gaisser, S.; Challis, I. R.; Carletti, I.; Wilkinson, B.; Gregory, M.; Prodromou, C.; Roe, S. M.; Pearl, L. H.; Boyd, S. M.; Zhang, M.-Q. Molecular characterization of macbecin as an Hsp90 inhibitor. *J. Med. Chem.* **2008**, *51*, 2853–2857, and references therein.
(17) Rascher, A.; Hu, Z.; Buchanan, G. O.; Reid, R.; Hutchinson, C. R. Insights into the biosynthesis of the benzoquinone ansamycins geldanamycin and herbimycin, obtained by gene sequencing and disruption. *Appl. Environ. Microbiol.* **2005**, *71*, 4862–4871.
(18) Rascher, A.; Hu, Z.; Viswanathan, N.; Schirmer, A.; Reid, R.; Nierman, W. C.; Lewis, M.; Hutchinson, C. R. Cloning and characterization of a gene cluster for geldanamycin production in *Streptomyces hygroscopicus* NRRL 3602. *FEMS Microbiol. Lett.* **2003**, *218*, 223–230.
(19) Compounds **11** and **13** were identified by LC-MS analysis. The genes present in the pathway and the determined mass are consistent with the predicted structure in each case such that **11** is 11-*O*-desmethyl-macbecin and **13** is 4,5-dihydromacbecin.
(20) Onodera, H.; Kaneko, M.; Takahashi, Y.; Uochi, Y.; Funahashi, J.; Nakashima, T.; Soga, S.; Suzuki, M.; Ikeda, S.; Yamashita, Y.; Rahayu, E. S.; Kanda, Y.; Ichimura, M. Conformational significance of EH21A1-A4, phenolic derivatives of geldanamycin, for Hsp90 inhibitory activity. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1577–1580.
(21) Ono, Y.; Kozai, Y.; Ootsu, K. Antitumor and cytocidal activities of a newly discovered benzenoid ansamycin, macbecin I. *Gann* **1982**, *73*, 938–944.
(22) Goolarish, L. T.; Fernandes, C.; Yan, K.; Zhang, H.; Grooms, M.; Johanson, K.; Sinnamon, R. H.; Kirkpatrick, R. B.; Kerrigan, J.; Lewis, T.; Arnone, M.; King, A. J.; Lai, Z.; Copeland, R. A.; Tummnio, P. J. A biochemical rationale for the anticancer effects of Hsp90 inhibitors: Slow, tight binding inhibition by geldanamycin and its analogues. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 7625–7630.
(23) Sydor, J. R.; Normant, E.; Pien, C. S.; Porter, J. R.; Ge, J.; Grenier, L.; Pak, R. H.; Ali, J. A.; Dembski, M. S.; Hudak, J.; Patterson, J.; Penders, C.; Pink, M.; Read, M. A.; Sang, J.; Woodward, C.; Zhang, Y.; Grayzel, D. S.; Wright, J.; Barrett, J. A.; Palombella, V. J.; Adams, J.; Tong, J. K. Development of 17-allyamino-17-demethoxygeldanamycin hydroquinone hydrochloride (IPI-504), and anti-cancer agent directed against Hsp90. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 17408–17413.
(24) Jez, J. M.; Chen, J. C.-H.; Rastelli, G.; Stroud, R. M.; Santi, D. V. Crystal structure and molecular modeling of 17-DMAG in complex with human Hsp90. *Chem. Biol.* **2003**, *10*, 361–368.
(25) Abad-Zapatero, C.; Metz, J. T. Ligand efficiency indices as guideposts for drug discovery. *Drug Discovery Today* **2005**, *10*, 464–469.

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