Search for Hsp90 Inhibitors with Potential Anticancer Activity: Isolation and SAR Studies of Radicicol and Monocillin I from Two Plant-Associated Fungi of the Sonoran Desert[1]

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In an effort to discover small molecule inhibitors of Hsp90, we have screened over 500 EtOAc extracts of Sonoran desert plant-associated fungi using a two-stage strategy consisting of a primary cell-based heat shock induction assay (HSIA) followed by a secondary biochemical luciferase refolding assay (LRA). Bioassay-guided fractionation of extracts active in these assays derived from *Chaetomium chiversii* and *Paraphaeosphaeria quadriseptata* furnished the Hsp90 inhibitors radicicol (**1**) and monocillin I (**2**), respectively. In SAR studies, **1**, **2**, and their analogues, **3**−**16**, were evaluated in these assays, and the antiproliferative activity of compounds active in both assays was determined using the breast cancer cell line MCF-7. Radicicol and monocillin I were also evaluated in a solid-phase competition assay for their ability to bind Hsp90 and to deplete cellular levels of two known Hsp90 client proteins with relevance to breast cancer, estrogen receptor (ER), and the type 1 insulin-like growth factor receptor (IGF-1R). Some inferences on SAR were made considering the crystal structure of the *N*-terminus of yeast Hsp90 bound to **1** and the observed biological activities of **1**−**16**. Isolation of radicicol and monocillin I in this study provides evidence that we have developed an effective strategy for discovering natural product-based Hsp90 inhibitors with potential anticancer activity.

Hsp90 is an abundant and evolutionarily conserved 90 kDa cellular protein that performs essential functions not just following heat stress, but also under basal, physiological conditions, where it is critical for the stability, maturation, activation, and degradation of numerous nuclear hormone receptors (e.g., estrogen receptor),[2] nonnuclear signaling proteins (e.g., receptor-linked tyrosine kinases),[2] and stabilization and folding of various oncogenic proteins (e.g., RAF1 and HER2).[3] Under stress conditions such as heat, proteins tend to denature, exposing hydrophobic patches that are bound by the chaperone machinery in a non-sequence-specific manner to prevent the toxic effects of protein aggregation and to facilitate protein folding and renaturation. The binding of Hsp90 to partially unfolded proteins appears to release the monomeric form of the heat shock transcription factor Hsf-1. Its release is followed by trimerization and translocation of Hsf-1 to the nucleus, where the transcription factor binds the heat shock response element (HSE) in the promoter region of all the major stress response genes including the inducible form of Hsp90 itself. A final phosphorylation step is required for transactivation and up-regulation of the heat shock response.[4] Given Hsp90's dual role in both the stress response and in maintaining regulatory signaling networks, it appears that Hsp90 plays a critical role in the cancer phenotype and may provide a particularly effective target for cancer chemotherapy.[5] Two main observations support this hypothesis. Cancer cells frequently express high levels of Hsp90, presumably in response to the stress conditions within the tumor microenvironment. Hsp90 is also known to stabilize multiple critical oncoproteins, or to in effect stabilize oncogenic signaling pathways. As a result, pharmacological inhibition of this single target by compounds such as geldanamycin (GDA; **22**) has been shown to simultaneously destabilize many of the substrates known to be critical for the process of multistep carcinogenesis.[6] With the validation of Hsp90 as an anticancer drug target supported by ongoing phase I and II clinical trials of 17-allylaminogeldanamycin (17-AAG; **23**),[7] a derivative of the microbial metabolite GDA (**22**), we have undertaken a multidisciplinary effort to search for inhibitors of Hsp90 from microorganisms living in close association with plants of the North American Sonoran desert. Contrary to common assumptions concerning biodiversity in arid regions,[8] we have found a surprisingly rich and diverse range of microorganisms to be associated with plant communities indigenous to this region. For the discovery of small molecule natural product inhibitors of Hsp90 with potential anticancer activity, we developed a strategy involving (i) a primary moderate-throughput phenotypic screen using the cellular heat shock response as monitored by enhanced green fluorescent protein (EGFP) expression as the endpoint (heat shock induction assay; HSIA) and (ii) a low-throughput but well-characterized secondary assay for direct inhibition of chaperone activity involving the ATP-dependent refolding of heat-denatured luciferase (luciferase-refolding assay; LRA).[9] These assays were used to identify active extracts and to guide their subsequent fractionation to yield pure compounds. Once isolated, compounds were identified and biochemically characterized for their ability to interact with Hsp90 using a solid-phase-immobilized GDA derivative.[10] Biological evaluation consisted of standard cytotoxicity testing against MCF-7 breast cancer cells[11] and immunoblotting of lysates from drug-treated MCF-7 cells to confirm induction of the heat shock response at the protein level and depletion of the known Hsp90 client proteins ER and IGF-1R.

Using this approach, we have evaluated EtOAc extracts derived from over 500 Sonoran desert plant-associated endophytic and rhizosphere fungal strains. Several extracts active in both primary and secondary assays were encountered. Two such extracts were derived from *Chaetomium chiversii* (Chaetomiaceae), endophytic on the stem tissue of Mormon tea (*Ephedra fasciculata* A. Nels.; Ephedraceae), and *Paraphaeosphaeria quadriseptata* (Montagnulaceae), inhabiting the rhizosphere of the Christmas cactus (*Opuntia leptocaulis* DC.; Cactaceae). HSIA-guided fractionation of the

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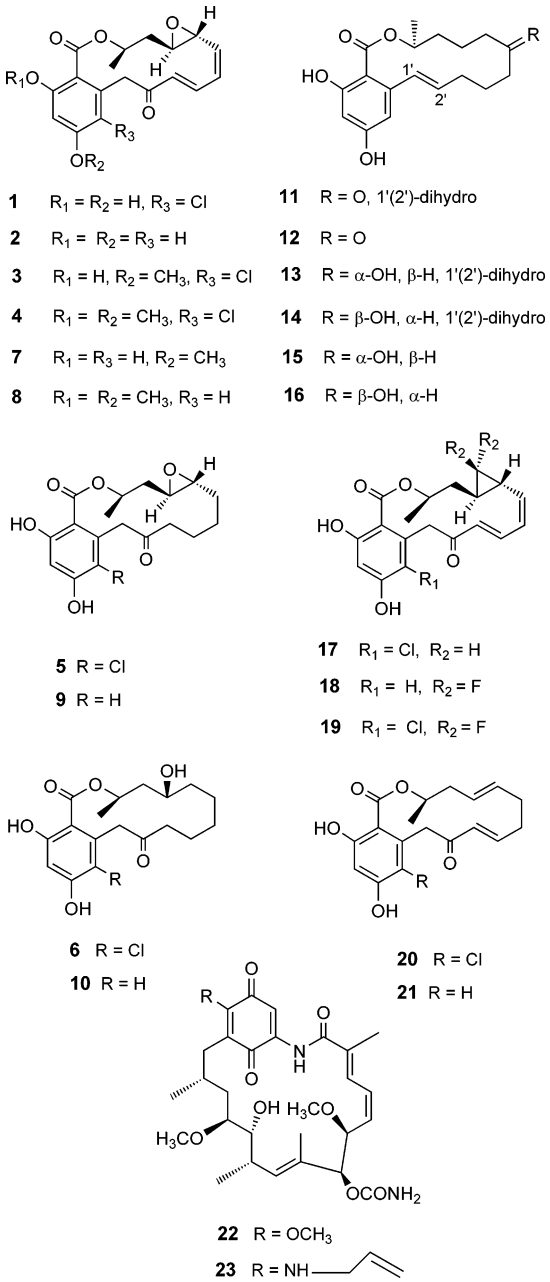

EtOAc extract of *C. chiversii* afforded the known Hsp90 inhibitor radicicol (**1**)[12] as the only active compound of this extract, and fractionation of the EtOAc extract of *P. quadriseptata* following a similar procedure yielded the resorcinylic macrolide monocillin I (**2**), structurally related to **1**. Although *C. chiversii* has not been subjected to any chemical investigation to date, previous studies of *P. quadriseptata* have led to the isolation of **2**,[13] which has also been found to occur in the fungal strain *Monocillium nordinii*.[14] Radicicol (**1**) has previously been encountered in the soil-borne fungi *Cylindrocarpon radicicola*,[12a,b] *Penicillium luteo-aurantium*,[12c] and *Humicola* sp. FO-2942.[12d] While radicicol displays Hsp90 inhibitory and in vitro anticancer activities, it was found to be devoid of any in vivo activity in animal models,[15] although some oxime derivatives of it were shown to possess in vivo efficacy.[16] A recent study has demonstrated that the synthetic analogue, cyclopropa-radicicol (**17**), in which the oxirane in **1** is replaced with a cyclopropane ring exhibits strong Hsp90 inhibitory activity and that the difluorocyclopropyl analogue **18** of monocillin I was capable of degrading the oncogenic protein HER2 at 1 μM, whereas the related radicicol analogue **19** was found to be less active and degraded HER2 at 10 μM.[17] However, in another recent investigation pochonin D (**20**) was found to be considerably more active than its nonchlorinated analogue **21** for affinity toward Hsp90 in a competition assay using GDA.[18] In a limited SAR study, **1** and **2**, their derivatives, **3**−**10**, and the commercially available zearalanone analogues, **11**−**16**, were evaluated for Hsp90 inhibitory activity in our primary and secondary assays. Those compounds active in these assays were tested for inhibition of proliferation of the breast cancer cell line MCF-7. This report constitutes the first evidence for the occurrence of radicicol (**1**) in an endophytic fungus and documents the Hsp90 inhibitory activity of monocillin I (**2**) and the analogues **3**, **5**, **9**, **12**, **14**, and **16**.

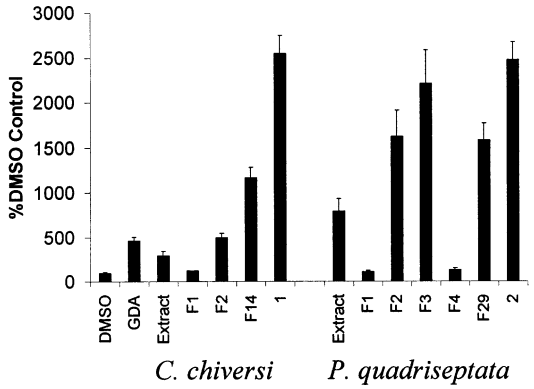

Figure 1. Cell-based heat shock induction assay (HSIA). The materials tested were DMSO (negative control), geldanamycin (GDA, positive control), EtOAc extracts, and major fractions, radicicol (**1**) and monocillin (**2**), derived from *C. chiversii* and *P. quadriseptata*. All samples except F29 were tested at 5 μg/mL; the concentration of F29 used was 2.5 μg/mL. The mean and standard deviation (SD) of triplicate determinations are presented, expressed as a percentage of the negative control; results are representative of three independent experiments.

Results and Discussion

Initial small-scale liquid−liquid partitioning[19] of the HSIA-active EtOAc extract of *C. chiversii* with hexane and 80% aqueous MeOH, followed by dilution of the latter fraction with water to 50% aqueous MeOH and extraction with $CHCl_3$, indicated that the activity was concentrated in the 80% aqueous MeOH fraction (F2; Figure 1, left). Further fractionation resulted in partitioning of the active compound(s) into both $CHCl_3$ and 50% aqueous MeOH. Therefore, the total 80% aqueous MeOH fraction (F2) was subjected to size exclusion chromatography on Sephadex LH-20 to obtain 20 combined fractions (F3−F22). The combined HSIA-active fraction (F14) was further fractionated by column chromatography over silica gel and repeated preparative TLC to furnish radicicol (**1**) as the only active compound. The structure of radicicol (**1**) was established by comparison of its physical and spectroscopic data with those reported in the literature.[12] Treatment of **1** with CH_3I and K_2CO_3 in acetone gave its new derivative, monomethyl radicicol (**3**), and dimethyl radicicol (**4**), previously obtained during the synthesis of radicicol analogues.[20] Catalytic hydrogenation of radicicol (**1**) afforded a mixture of tetrahydroradicicol (**5**)[12e] and hexahydroradicicol (**6**).[12e] The HSIA-active EtOAc extract of the rhizosphere fungus *P. quadriseptata* was partitioned with hexane and 80% aqueous MeOH. The bioactive aqueous MeOH fraction (F2; Figure 1, right) was diluted to 60% aqueous MeOH by the addition of water and extracted with $CHCl_3$. Size exclusion chromatography of the HSIA-active $CHCl_3$ fraction (F3) on a column of Sephadex LH-20, followed by silica gel column chromatography of the combined active fraction (F29), yielded monocillin I (**2**) as the only active compound of this extract. The analogues of **2**, namely, monomethyl monocillin I (**7**), dimethyl monocillin I (**8**), tetrahydromonocillin I (**9**), and hexahydromonocillin I (**10**), were prepared as described previously.[13]

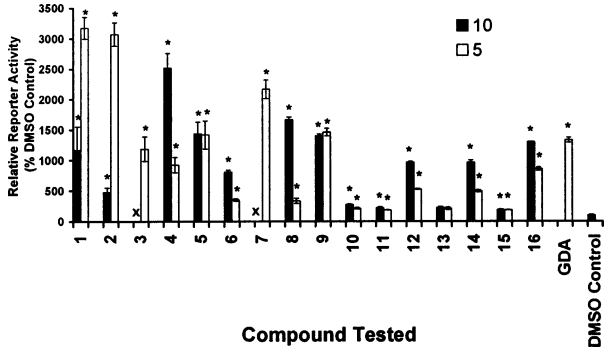

Figure 2. Cell-based heat shock induction assay (HSIA) of compounds **1**−**16** at 10.0 and 5.0 μM, GDA (positive control) at 5.0 μM, and DMSO (negative control). Reporter activity in wells treated with test compounds relative to DMSO control is depicted. × indicates that no living cells were found in the wells treated with these compounds at 10 μM. The mean and SD of triplicate determinations are presented, expressed as a percentage of the negative control; results are representative of three independent experiments. An asterisk (*) indicates significant statistical difference of DMSO and the test condition (p value of <0.05) in a pooled-variance two-sample T-test.

Compounds **3**−**10** and the commercially available structural analogues zearalanone (**11**), zearalenone (**12**), α-zearalanol (**13**), β-zearalanol (**14**), α-zearalenol (**15**), and β-zearalenol (**16**) were evaluated for their heat shock induction activity at concentrations of 10.0 and 5.0 μM. As shown in Figure 2, radicicol (**1**), monocillin I (**2**), monomethyl radicicol (**3**), and monomethyl monocillin I (**7**) showed considerably lower activity at 10.0 μM compared with their activity at 5.0 μM. Microscopic evaluation of treated wells indicated that these compounds were toxic to the reporter 3T3-Y9/B12 cells used in our HSIA at a concentration of 10.0 μM. Therefore, comparison of activity in HSIA was made using data at 5.0 μM concentration, although this may not be the optimum concentration for maximal heat shock induction by some of the analogues tested. HSIA results indicated that of the 16 compounds tested, radicicol (**1**), monocillin I (**2**), monomethyl radicicol (**3**), dimethyl radicicol (**4**), tetrahydroradicicol (**5**), monomethyl monocillin I (**7**), and tetrahydromonocillin I (**9**) had comparable or higher activity, whereas dimethyl radicicol (**4**), zearalenone (**12**), β-zearalanol (**14**), and β-zearalenol (**16**) exhibited slightly reduced activity than the positive control (GDA; **22**) at the same concentration (Figure 2). Compounds **1**−**16** were also evaluated in LRA for inhibitory activity against chaperone-mediated refolding of the heat-denatured enzyme luciferase. As depicted in Figure 3A, radicicol (**1**), monocillin I (**2**), monomethyl radicicol (**3**), tetrahydoradicicol (**5**), tetrahydromonocillin I (**9**), zearalenone (**12**), β-zearalanol (**14**), and β-zearalenol (**16**) were found to be approximately as active as the positive control (GDA) in this secondary assay. It is noteworthy that the compounds demonstrating statistically significant activity compared to DMSO control in the HSIA also showed significant activity in the LRA except for dimethyl radicicol (**4**) and monomethyl monocillin I (**7**), which consistently showed moderate activity in the HSIA but were not active in the LRA. The reason for the discrepancy with these compounds is not clear, but could involve cellular metabolism since the HSIA is performed in living cells, while the LRA is not. A time course study (Figure 3B) showed that, as expected, radicicol (**1**) and monocillin I (**2**) retarded the luciferase refolding in a concentration- and time-dependent manner. These findings suggest that compounds **1**−**3**, **5**, **7**, **9**, **12**, **14**, and **16** are capable of disrupting chaperone-mediated refolding of denatured luciferase. On the basis of their structural homology with radicicol (**1**), we predicted that these would also interact as ADP mimetics with the *N*-terminal nucleotide binding site of Hsp90.[21] To test this, we examined the ability of **2** to compete for binding

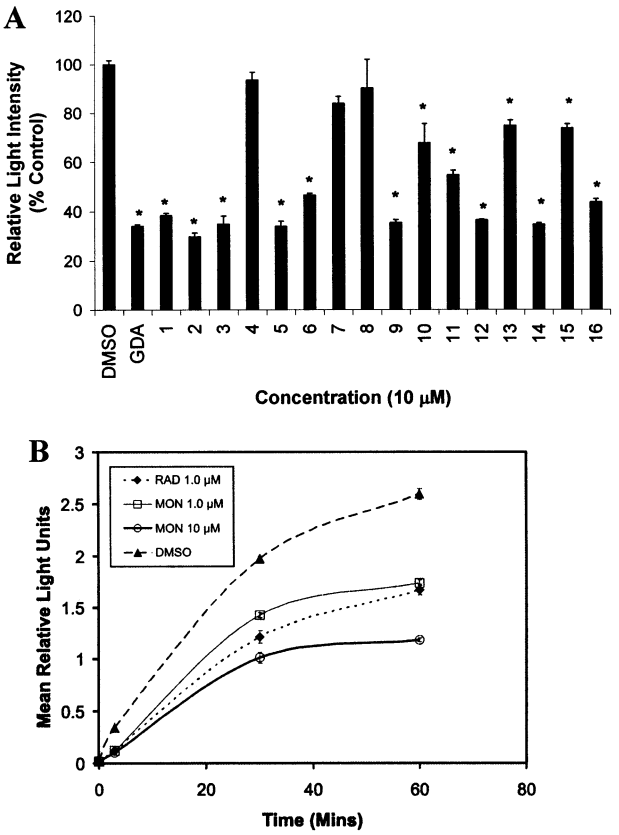

Figure 3. (A) Inhibition of heat-denatured luciferase renaturation (luciferase-refolding assay; LRA) of compounds **1**−**16**, GDA (positive control), and DMSO (negative control) after 3 min incubation at 28 °C. The mean and SD of triplicate determinations are presented, expressed as a percentage of the negative control; results are representative of three independent experiments. An asterisk (*) indicates significant statistical difference of DMSO and the test condition (p value of <0.05) in a pooled-variance two-sample T-test. (B) Time course of inhibition of renaturation of heat-denatured luciferase by radicicol [RAD (**1**)] at 1.0 μM and monocillin I [MON (**2**)] at 1.0 and 10.0 μM. The mean and SD of triplicate determinations are presented; results are representative of three independent experiments. Analysis of variance (ANOVA), analysis of covariance (ANCOVA), and standard post hoc tests (e.g., Tukey test) were performed on these data. ANCOVAs demonstrated significant differences between the curves of DMSO and the other test conditions. Post hoc tests showed at time zero there were no differences among the groups. At 3 min, DMSO was significantly different from the other compounds ($p < 0.0001$); MON at 1 μM and at 10 μM were not significantly different from one another ($p = 0.088$). At 30 min, DMSO was significantly different from the other compounds ($p < 0.0001$); MON at 1 μM also was significantly different from MON at 10 μM ($p < 0.0001$). At 60 min, DMSO was significantly different from the other compounds ($p < 0.0001$); MON at 1 μM also was significantly different from MON at 10 μM ($p < 0.0001$).

Hsp90 in cell lysate with a bead-immobilized derivative of the classical Hsp90 inhibitor geldanamycin (GDA; **22**). As shown in Figure 4A, radicicol (**1**) and monocillin I (**2**) were able to displace the Hsp90 bound to GDA-derivatized beads in a concentration-dependent manner. Inhibition was comparable to that of GDA, suggesting that both **1** and **2** interact with the same nucleotide-binding site in the amino terminus of Hsp90 known to be targeted by GDA.

It is known that Hsp90 is required for the stability, maturation, and activation of various receptor and signaling proteins including ER and IGF-1R.[22] Drug-mediated inhibition of Hsp90 leads to degradation of many of its client proteins via the ubiquitin−

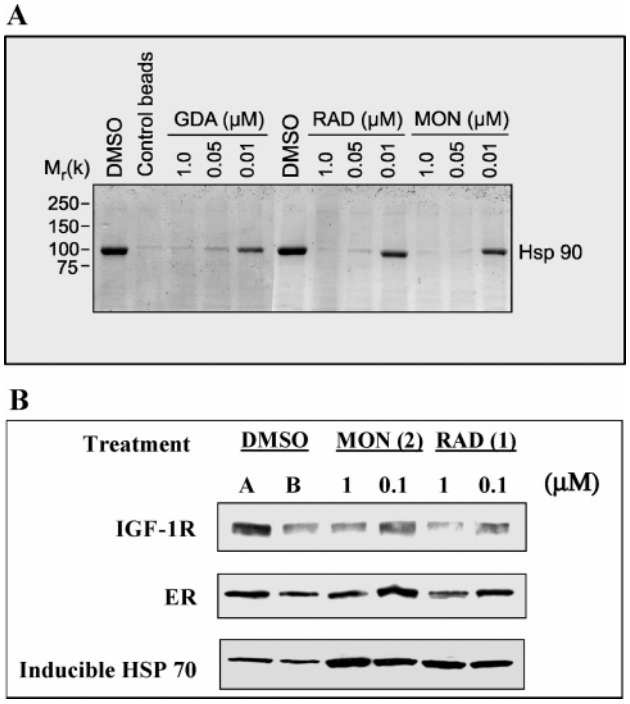

Figure 4. (A) GDA-immobilized bead competition assay of radicicol (**1**) and monocillin I (**2**). Soluble radicicol [RAD (**1**)] and monocillin I [MON (**2**)] compete for the binding of Hsp90 in cell lysate to the solid phase-immobilized derivative of GDA. (B) Immunoblot demonstrating depletion of the Hsp90 client protein estrogen receptor (ER) and type 1 insulin-like growth factor receptor (IGF-1R), and induction of the expression of Hsp70 by RAD (**1**) and MON (**2**). Results are representative of three independent experiments. DMSO lane B was loaded with half the amount of the total protein of lane A.

proteosome pathway.[23] Additionally, inhibition of Hsp90 leads to up-regulation of heat shock protein genes such as *HSP70*, and it was this activity that provided the rationale for development of our primary screen (HSIA).[24] We therefore confirmed the ability of radicicol (**1**) and monocillin I (**2**) to cause the degradation of Hsp90 client proteins ER and IGF-1R and the induction of the heat shock protein HSP72. For this purpose, MCF-7 breast cancer cells were treated overnight with **1** and **2** at concentrations of 1.0 and 0.1 μM, after which cell lysates were prepared and immunoblotted with antibodies to IGF-1R, ER, and Hsp72, a highly inducible isoform of the Hsp70 family. As expected, these compounds were found to deplete cellular levels of the Hsp90 client proteins ER and IGF-1R in MCF-7 cells (Figure 4B). In addition, Hsp72 was found to be robustly up-regulated (Figure 4B). The compounds **1**−**5**, **7**, **9**, **12**, **14**, and **16**, which were positive in our primary and/or secondary assays for heat shock activity, were also evaluated for their antiproliferative activity against the breast cancer cell line MCF-7 using an MTT assay.[11] Interestingly, only radicicol (**1**), monocillin I (**2**), monomethyl radicicol (**3**), and tetrahydroradicicol (**5**) were found to inhibit the proliferation/survival of MCF-7 cells in the sub-micromolar concentration range, with IC_{50} values of 0.03, 0.34, 0.90, and 0.19 μM, respectively; others did not show any detectable inhibition at concentrations up to 1.00 μM.

Roe et al.[21] have reported the crystal structure for the *N*-terminus of yeast Hsp90 bound to radicicol (**1**) and ADP. In this crystal structure, **1** is positioned and configured in such a way as to mimic the nucleotide (ADP) in the unusual hydrophobic ATPase binding pocket of Hsp90. Since the small molecule inhibitors of Hsp90 are known to bind this pocket with higher affinity than the nucleotide, the effect is to lock Hsp90 in a conformation with similarities to its ADP-bound state. The activities of Hsp90 in this conformation are thus favored, including high substrate affinity with increased degradation of client proteins and release of Hsf-1 and the consequent activation of the stress response.[25] In the case of radicicol (**1**), the aromatic ring is approximately coplanar with the adenine ring of ADP and with the Cl atom on the aromatic ring of **1** in van der Waals contact with Phe124 of the protein.[21] There are no apparent conformational changes in the *N*-terminal domain or reordering of side chains as a result of radicicol binding. Our efforts to use this model to explain the biological activities observed for radicicol (**1**), monocillin I (**2**), and their analogues **3**−**16** resulted in the following plausible structure−activity relationships: (i) Monocillin I (**2**) was found to be as active as radicicol (**1**) in both HSIA and LRA; thus, it appears that the Cl atom at C-5 is not essential for Hsp90 activity. However, **2** was found to be about 10-fold less cytotoxic than **1**, suggesting that C-5-Cl may contribute to the enhanced cytotoxicity of **1**. (ii) Reduction of the conjugated diene at C-3′(4′)−C-5′(6′) of the macrocyclic lactone ring system as exemplified by **5** and **9** does not appear to impact either heat shock induction or protein-refolding activity. However, tetrahydromonocillin I (**9**) was found to be noncytotoxic at 1.0 μM (see below); the loss of cytotoxicity with retention of HSIA activity and biochemical inhibition of Hsp90 chaperone function suggests that either the ability of this compound to enter the cell or its subsequent stability and/or metabolism might be altered. Alternatively, these functional groups might be responsible for non-target-related activities of these compounds, and removal of these could make them more specific to Hsp90 (see below). (iii) The opening of the oxirane ring [at C-7′−C-8′] of **5** and **9** resulting in **6** and **10**, respectively, leads to the loss of all three activities. However, the presence of β-OH at C-6′ as in β-zearalenol (**16**) leads to the moderate retention of both HSIA and LRA activities. In the crystal structure the oxirane is oriented out of the Hsp90 binding pocket and participates in a hydrogen bond with Lys 44,[21] and it is possible that the C-6′-β-OH in **16** plays a similar role. Moderate HSIA activity of zearalenone (**12**) and β-zearalanol (**14**), while supporting this, also suggests the importance of the sp^2-hybridized carbon at C-2′ of the macrocyclic ring for heat shock induction. (iv) The hydroxyl groups at C-2 and C-4 of the aromatic ring contribute unequally to the activities of **1** and **2**, since in the case of monomethyl radicicol (**3**) and monomethyl monocillin I (**7**) heat shock induction activity was retained, but in the case of dimethyl radicicol (**4**) and dimethyl monocillin I (**8**) this activity was considerably reduced or lost. Both hydroxyl groups are known to participate in hydrogen bonding with amino acid residues within the Hsp90 binding site,[21] suggesting that in the case of radicicol (**1**) the more distal hydroxyl group from the Cl atom carries out a critical role in the stabilization of radicicol (**1**) in the binding site. (v) Heat shock induction and luciferase-refolding activities of **12**, **14**, and **16** suggest that the carbon skeleton (macrocyclic lactone ring system) of these compounds contributes to their activity, and more importantly the presence of sp^2-hybridized C-2′ and/or a β-oriented oxygen atom in the vicinity of C-6′−C-8′ are important for the observed Hsp90 inhibitory activity. These partial structure−activity relationships for radicicol (**1**) are summarized in Figure 5.

The antiproliferative activity of these compounds seems to reflect their activity in the HSIA and LRA except for tetrahydromonocillin I (**9**), zearalenone (**12**), β-zearalanol (**14**), and β-zearalenol (**16**), which were active in these assays, but did not inhibit the growth of MCF-7 cells effectively. The lack of cytotoxic activity in the nonchlorinated analogue **9** may be due to the absence of the enone moiety that may be essential for this activity, as is the case with many cytotoxic compounds.[26] This discrepancy in the case of **12**, **14**, and **16** may be due to the estrogenic properties of these compounds[27] and our use of the MCF-7 cell line, which is known to be estrogen receptor positive. In fact, a recent study found that zearalenone (**12**), β-zearalanol (**14**), and β-zearalenol (**16**) stimulated proliferation of MCF-7 cells relative to a non-estrogen-sensitive breast cancer cell line, MDA-MB-231.[28] The fact that after exposure

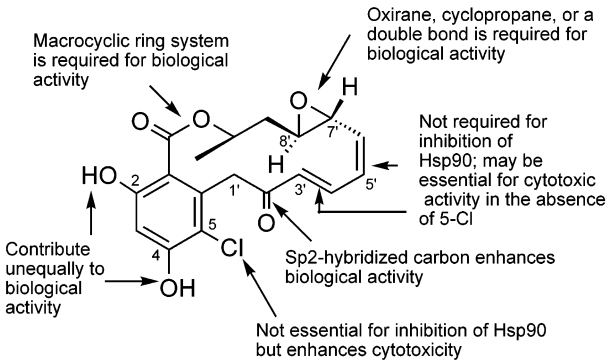

Figure 5. Partial structure−activity relationships for radicicol (**1**).

to **1** and **2** Hsp90 client proteins IGF-1R and ER are degraded in MCF-7 cells and that **1** and **2** were able to effectively compete with immobilized GDA for Hsp90 binding further support the concept that these molecules are exerting their antiproliferative effects by directly interacting with Hsp90. The isolation of the macrocyclic β-resorcylic acid lactones radicicol (**1**) and monocillin I (**2**) using the above approach and our ability to define structure−activity relationships for small molecules that target Hsp90 based on the outcome of the assays employed in this study provide evidence that we have developed an effective strategy for the discovery of natural product-based inhibitors of Hsp90 chaperone function. Further studies are in progress to search for additional small molecule natural product inhibitors of Hsp90 and to optimize the anticancer activity of macrocyclic β-resorcylic acid lactones.

Experimental Section

General Experimental Procedures. Melting points were determined on an electrothermal melting point apparatus and are uncorrected. Optical rotations were measured with a Jasco Dip-370 polarimeter using $CHCl_3$ or MeOH as solvent. IR spectra for KBr disks were recorded on a Shimadzu FTIR-8300 spectrometer. NMR spectra were recorded in $CDCl_3$ or acetone-d_6 with a Bruker DRX-600 instrument at 600 MHz for ^{1}H NMR, and a Bruker DRX-500 instrument at 125 MHz for ^{13}C NMR, respectively, using residual $CHCl_3$ and acetone as internal standards. The chemical shift values (δ) are given in parts per million (ppm), and the coupling constants are in Hz. Low- and high-resolution MS were recorded, respectively, on Shimadzu LCMS-8000 QPα and JEOL HX110A spectrometers. Geldanamycin (GDA) was purchased from Alexis Biochemicals (San Diego, CA). All other reagents and chemicals including zearalanone, zearalenone, α-zearalanol, β-zearalanol, α-zearalenol, and β-zearalenol were purchased from Sigma unless otherwise noted. The heat shock reporter cell line, 3T3-Y9/B12, was constructed by stably transfecting NIH-3T3 with a plasmid encoding EGFP under the control of a minimal heat shock response element derived from the promoter region of the human *Hsp70B* gene (gift of T. Tsang, University of Arizona). Rabbit reticulocyte lysate (RRL) was purchased from Green Hectares Farm (Oregon, WI) and stored in liquid nitrogen until use. Goat-anti-mouse and rabbit-secondary antibodies conjugated to horseradish peroxidase for chemiluminescence detection were purchased from Kiekegaard and Perry Laboratories, Inc. (Gaithersburg, MD). Monoclonal antibodies to Hsp70 (SPA810), insulin growth factor 1 receptor (IGF-1R; C-20), and estrogen receptor (ER; AB-14) were purchased from Stressgen (Victoria, British Columbia, Canada), Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), and Neomarkers, Inc. (Fremont, CA), respectively.

Fungal Isolation and Identification. The endophytic fungal strain was isolated from the stems of *Ephedra fasciculata* growing in South Mountain Park in Phoenix, Arizona, and was identified by Microbial ID Inc., Newark, DE, as *Chaetomium chiversii*. The strain is deposited in the School of Life Sciences, Arizona State University, and Southwest Center for Natural Products Research and Commercialization (SCNPRC) of the University of Arizona microbial culture collections under the accession numbers 7-EPH-2S and CS-36-62, respectively. The rhizosphere fungal strain occurring on the surface of the roots of *Opuntia leptocaulis* was isolated and identified as *Paraphaeosphaeria quadriseptata*, as described previously.[13] The strain is deposited in the Division of Plant Pathology, Department of Plant Sciences, and SCNPRC of the University of Arizona microbial culture collections under the accession number AH-45-00-F20. Both microorganisms were subcultured using Petri dishes with PDA, and for long-term storage, isolates were subcultured on PDA slants, overlaid with 40% glycerol, and stored at −80 °C in our library of the Sonoran desert plant-associated microorganisms.

Heat Shock Induction Assay (HSIA). 3T3-Y9/B12 reporter cells were seeded into flat-bottomed 96-well plates (Falcon; Becton Dickinson, Lincoln Park, NJ) at a density of 20 000 cells/well and allowed to attach overnight (one column of wells was left empty to serve as blank controls). On the following day, serial 2-fold dilutions of pure compounds or single dilutions of extracts or fractions were added in triplicate to cell-containing wells. Triplicate wells were also treated with DMSO vehicle alone (volume not to exceed 0.1%) to serve as a negative control. Cells were incubated overnight, the medium was removed, and wells were rinsed once with PBS, followed by addition of 150 μL of PBS to each well. Fluorescence was determined on a Fluoroskan (LabSystems) or Analyst AD (LJL Biosystems) plate reader equipped with filter sets for excitation at 485 nm and emission at 525 nm. Mean florescence and standard deviations of triplicate determinations were calculated and plotted.

Luciferase Refolding Assay. Rabbit reticulocyte lysate (RRL) was supplemented with an ATP regeneration system (10 mM phosphocreatine, 1 mM ATP, and 17.5 units of creatine phosphokinase) as previously described.[9] Test compounds formulated in DMSO or an equal volume of DMSO vehicle alone were then added to a 100 μL aliquot of RRL in a thin-walled Eppendorf tube and allowed to equilibrate for 20 min. Firefly luciferase (100 nM) in stability buffer (SB: 25 mM tricine-HCl, pH 7.8; 8 mM $MgSO_4$; 0.1 mM EDTA; 10 mg/mL bovine serum albumin; 10% glycerol; and 0.25% Triton-X100) was heat-denatured for 8 min at 40 °C and placed on ice for 10 min. Another tube of 100 nM firefly luciferase in SB was maintained in its native state on ice to serve as a control. Native or heat-denatured firefly luciferase was diluted 10-fold into RRL containing the various test compounds and placed on ice. At starting time 0, triplicate 5 μL aliquots were transferred from each reaction tube to a cooled white-walled 96-well plate and assayed for luciferase activity by addition of 100 μL assay buffer (25 mM tricine-HCL, pH 7.8; 8 mM $MgSO_4$; 0.1 mM EDTA; 12 mM dithioreitol; 100 μM luciferin; 240 μM CoA; and 0.5 mM ATP) using the automated injector on a Fluroskan plate reader set to luminometry mode. After time 0, the reaction tubes were incubated continuously at 28 °C in a PCR thermocyler, and aliquots were removed and assayed as above at 3, 30, and 60 min after addition of the heat-denatured enzyme. The effects of drug treatments on the activity of native luciferase were assayed at 0 and 60 min to ensure that the compounds did not directly inhibit luciferase activity. Mean relative light units (RLU) were calculated for each time point and condition.

Cytotoxicity Assay. MCF-7 cells were seeded into 96-well plates at a density of 2000 cells/well. The following day, serial dilutions of test compounds were added to triplicate wells. GDA was used as a positive control, and an equal volume of DMSO vehicle served as a negative control. Cells were incubated under 5% CO_2 at 37 °C for 3 days followed by addition of MTT [1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan].[11] After incubation in the dark at 37 °C for 3 h, medium was removed and 200 μL/well of DMSO was added. Plates were gently agitated for 5 min, and absorbance was read at 570 nm with background subtracted at 650 nm.

Solid-Phase Hsp90 Binding Assay. Geldanamycin (GDA) was derivatized and immobilized on agarose beads as previously reported.[10] Aliquots of MCF-7 cell lysate were incubated with GDA-coupled beads with or without previous addition of soluble compounds at various concentrations or an equal volume of DMSO solvent vehicle. After tumbling for 60 min in the cold, the beads were washed four times in nonionic detergent buffer. Bound proteins were eluted by heating in reducing loading buffer and fractionated by SDS-PAGE followed by visualization using Coomassie stain.

Western Blotting. MCF-7 cells were seeded in 6-well plates at a density of 5×10^5 cells/well and allowed to attach overnight. The following day, cells were treated with 1.0 and 0.1 μM of the test compounds or an equal volume of DMSO vehicle (the final concentration of DMSO in all treatments did not exceed 0.1%). Cells were incubated overnight and then lysed in cold TNES buffer (50 mM Tris

HCl, pH 7.4; 1% Igepal; 2 mM EDTA; 100 mM NaCl; 1 mM phenylmethylsulfonyl fluoride; 20 μg/mL leupeptin; and 20 μg/mL aprotinin). Lysates were clarified by centrifugation at 14 000 rpm for 15 min, and the protein content of the supernatants was determined by bicinchonic acid assay (BCA; Pierce Biotechnology, Rockford, IL) using bovine serum albumin (BSA) as a standard. Equal amounts of total protein from DMSO- and drug-treated cells were fractionated by SDS-PAGE on a 7.5% polyacrylamide gel and transferred to nitrocellulose. The membrane was stained with the reversible dye Ponceau Red to verify equal loading. Membranes were subsequently blocked in 3% nonfat dry milk in Tris-buffered saline with 0.05% Tween 20 for 30 min and probed for Hsp70, ER, or IGF-1R with primary antibodies at 1 to 100 dilutions overnight at 4 °C. Signals were detected by chemiluminescence using the appropriate secondary antibodies conjugated to horseradish peroxidase and Supersignal substrate (Pierce). Multiple exposures were obtained to ensure that the signals were in the dynamic range of the film.

Cultivation and Isolation of Radicicol from *Chaetomium chiversii.* For isolation of secondary metabolites, the fungus was cultured in 120 T-flasks (800 mL), each containing 135 mL of PDA coated on five sides of the flasks, maximizing the surface area for fungal growth (total surface area/flask ca. 460 cm^2). After incubation for 14 days at 27 °C, MeOH (200 mL/T-flask) was added to all 120 T-flasks and shaken in a rotary shaker for 12 h at 27 °C, and the resulting extract was filtered through Whatman No. 1 filter paper and a layer of Celite 545. The filtrate was concentrated to one-fourth of its original volume and extracted with EtOAc (4 × 1950 mL). Combined EtOAc extracts were evaporated under reduced pressure to afford a dark green semisolid (2.01 g), a portion (1.75 g) of which was partitioned between hexane and 80% aqueous MeOH. Evaporation of solvents under reduced pressure yielded hexane (F1; 0.535 g) and 80% aqueous MeOH (F2; 1.031 g) fractions. A portion (1.0 g) of the 80% aqueous MeOH fraction (F2) was subjected to size exclusion chromatography on a column of Sephadex LH-20 (30 g) in hexane/CH_2Cl_2 (1:4) and eluted with hexane/CH_2Cl_2 (1:4) (800 mL), CH_2Cl_2/acetone (3:2) (500 mL), CH_2Cl_2/acetone (1:4) (300 mL), CH_2Cl_2/MeOH (1:1) (300 mL), and finally MeOH (500 mL). Seventy fractions (20 mL each) were collected and pooled on the basis of their TLC profiles to yield 20 combined fractions (F3−F22). The combined fraction F14 (288 mg) active in HSIA was chromatographed over a column of silica gel (Fluka G 60, 6 g) made up in CH_2Cl_2 and eluted with CH_2Cl_2 containing increasing amounts of MeOH. Eighty fractions (4 mL each) were collected, and fractions having similar TLC patterns were combined to give 8 subfractions. The subfraction eluted with 0.5% MeOH on evaporation gave a pale yellow semisolid with significant activity in HSIA. This was dissolved in CH_2Cl_2 and triturated with hexane to afford radicicol (**1**) as a colorless solid (102 mg).

Cultivation and Isolation of Monocillin I from *Paraphaeosphaeria quadriseptata.* The fungus was cultured as for *C. chiversii* described above, but using 40 T-flasks. After 28 days at 27 °C, MeOH was added to each of the 40 T-flasks and allowed to soak overnight at room temperature, and the resulting extract was filtered through Whatman No. 1 filter paper and a layer of Celite 545. The filtrate was concentrated to one-fourth of its original volume and extracted with EtOAc (5 × 500 mL). Evaporation under reduced pressure afforded the EtOAc extract (1.85 g), which was found to be active in HSIA. A portion of this extract (1.80 g) was partitioned between hexane and 80% aqueous MeOH, yielding the fractions F1 and F2, respectively. The HSIA-active aqueous MeOH fraction (F2) was diluted to 60% aqueous MeOH with water and extracted with $CHCl_3$. Of the resulting fractions, only the $CHCl_3$ fraction (F3, 1.30 g) was found to be active (Figure 1), and this was subjected to size exclusion chromatography on a column of Sephadex LH-20 (40.0 g) made up in hexane/CH_2Cl_2 (1:4) and eluted with hexane/CH_2Cl_2 (1:4) (700 mL), CH_2Cl_2/acetone (3:2) (250 mL), CH_2Cl_2/acetone (1:4) (250 mL), and finally MeOH (250 mL). Twenty-four fractions (50 mL each) were collected (F5−F28), of which fractions F5−F21 were found to be active in HSIA. Fractions F5−F21 were combined, and the combined fraction F29 (790.4 mg) was further fractionated on silica gel (13.0 g) by elution with CH_2Cl_2 followed by increasing amounts of MeOH in CH_2Cl_2. Fractions eluted with 0.5% MeOH in CH_2Cl_2 were found to be HSIA-active, and these were combined and evaporated to yield monocillin I (**2**) (521 mg).

Radicicol (1): colorless crystals; mp 196 °C (lit.[12] 195 °C); $[\alpha]_D^{27}$ 187 (*c* 1.0, $CHCl_3$); ^{1}H NMR data were consistent with literature values;[12] APCIMS (+)ve mode *m*/*z* 365 $[M + H]^+$.

Monocillin I (2): white solid; mp 129−132 °C; $[\alpha]_D^{27}$ −34 (*c* 1.0, $CHCl_3$); physical and spectral (^{1}H and ^{13}C NMR) data were consistent with literature values;[14] APCIMS (+)ve mode *m*/*z* 331 $[M + H]^+$.

Methylation of Radicicol. To a solution of radicicol (15 mg) in acetone (0.5 mL) were added K_2CO_3 (20 mg) and CH_3I (0.3 mL), and the mixture was stirred at room temperature. After 45 min (TLC control), the reaction mixture was filtered and the filtrate evaporated and purified by preparative TLC (silica gel) using CH_2Cl_2 as eluant to give **3** (2.9 mg) and **4** (12.2 mg).

Monomethyl radicicol (3): white crystalline solid; mp 182−183 °C; IR (KBr) γ_{max} 3425, 1659, 1601, 1438, 1354, 1308, 1242, 1115 cm^{-1}; ^{1}H NMR (acetone-d_6, 600 MHz) δ 10.53 (1H, brs, OH-18), 7.48 (1H, dd, J = 16.1, 9.4 Hz, H-8), 6.64 (1H, s, H-15), 6.26 (1H, td, J = 9.4, 1.8 Hz, H-7), 6.10 (1H, d, J = 16.1 Hz, H-9), 5.74 (1H, dd, J = 9.4, 3.4 Hz, H-6), 5.45 (1H, m, H-2), 4.46 (1H, d, J = 16.3 Hz, H-11a), 3.91 (3H, s, OCH_3-14), 3.89 (1H, d, J = 16.3 Hz, H-11b), 3.29 (1H, m, H-5), 3.03 (1H, dt, J = 9.7, 2.7 Hz, H-4), 2.44 (1H, dt, J = 14.9, 3.4 Hz, H-3a) 1.79 (1H, ddd, J = 14.9, 8.7. 3.7 Hz, H-3b), 1.56 (3H, d, J = 6.6 Hz, CH_3-1); HRFABMS *m*/*z* $[M + H]^+$ 379.8178 (calcd for $C_{19}H_{20}ClO_6$, 379.8172).

Dimethyl radicicol (4): white crystalline solid; mp 186−188 °C; IR (KBr) γ_{max} 3400, 1724, 1651, 1596, 1466, 1339, 1269, 1215, 1083 cm^{-1}; ^{1}H NMR (acetone-d_6, 600 MHz) δ 7.45 (1H, dd, J = 15.7, 9.7 Hz, H-8), 6.86 (1H, s, H-15), 6.23 (1H, dd, J = 10.5, 9.7 Hz, H-7), 6.10 (1H, d, J = 15.7 Hz, H-9), 5.67 (1H, dd, J = 10.5, 4.9 Hz, H-6), 5.31 (1H, m, H-2), 3.94 (3H, s, OCH_3-16), 3.92 (1H, d, J = 15.7 Hz, H-11a), 3.91 (3H, s, OCH_3-14), 3.75 (1H, d, J = 15.7 Hz, H-11b), 3.39 (1H, m, H-5), 3.10 (1H, dt, J = 8.8, 3.7 Hz, H-4), 3.11 (1H, dt, J = 9.7, 2.7 Hz, H-4), 2.44 (1H, dt, J = 14.5, 3.4 Hz, H− 3a) 1.79 (1H, ddd, J = 14.9, 8.7. 3.7 Hz, H-3b), 1.56 (3H, d, J = 6.6 Hz, CH_3-1); APCIMS (+)ve mode *m*/*z* 393 $[M + H]^+$.

Hydrogenation of Radicicol. To a solution of radicicol (3 mg) in EtOH (1.0 mL) was added 10% Pd on carbon (1.0 mg). After hydrogenation for 10 min at room temperature (23 °C) under atmospheric pressure, the catalyst was removed by filtration and the residue of the filtrate was separated on preparative TLC (silica gel) using 6% MeOH in CH_2Cl_2 as eluant to give **5** (1.4 mg) and **6** (1.6 mg).

Tetrahydroradicicol (5): white crystalline solid; mp 176−178 °C; ^{1}H NMR data were consistent with literature values;[12e] APCIMS (+)-ve mode *m*/*z* 369 $[M + H]^+$.

Hexahydroradicicol (6): white crystalline solid; mp 181−183 °C; ^{1}H NMR data were consistent with literature values;[12e] APCIMS (+)-ve mode *m*/*z* 371 $[M + H]^+$.

Monomethyl Monocillin I (7), Dimethyl Monocillin I (8), Tetrahydromonocillin I (9), and Hexahydromonocillin I (10). Conversion of monocillin I (**2**) to its derivatives **7**−**10** and their spectroscopic characterization have been reported previously.[13]

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References and Notes

(1) Studies on Arid Lands Plants and Microorganisms, Part 7. For Part 6, see: Bashyal, B. P.; Wijeratne, E. M. K.; Faeth, S. H.; Gunatilaka, A. A. L. *J. Nat. Prod.* **2005**, *68*, 724−728.
(2) Csermely, P.; Schnaider, T.; Soti, C.; Prohaszka, Z.; Nardai, G. *Pharmacol. Ther.* **1998**, *79*, 129−168.
(3) Banerji, U.; Judson, I.; Workman, P. *Curr. Cancer Drug Targets* **2003**, *3*, 385−390.
(4) (a) Guo, Y.; Guettouche, T.; Fenna, M.; Boellmann, F.; Pratt, W. B.; Toft, D. O.; Smith, D. F.; Voellmy, R. *J. Biol. Chem.* **2001**, *276*, 45791−45799. (b) Bagatell, R.; Paine-Murrieta, G. D.; Taylor, C. W.; Pulcini, E. J.; Akinaga, S.; Benjamin, I. J.; Whitesell, L. *Clin. Cancer Res.* **2000**, *6*, 3312−3318.
(5) (a) Whitesell, L.; Lindquist, S. L. *Nat. Rev. Cancer* **2005**, *5*, 761−772. (b) Neckers, L. *Trends Mol. Med.* **2002**, *8*, S55−61. (c) Maloney, A.; Workman, P. *Expert Opin. Biol. Ther.* **2002**, *2*, 3−24.
(6) Bagatell, R.; Whitesell, L. *Mol. Cancer Ther.* **2004**, *3*, 1021−1030.

(7) Banerji, U.; O'Donnel, A.; Scurr, M.; Pacey, S.; Stapleton, S.; Asad, Y.; Simmons, L.; Maloney, A.; Raynaud, F.; Campbell, M.; Walton, M.; Lakhani, S.; Kaye, S.; Workman, P.; Judson, I. *J. Clin. Oncol.* **2005**, *23*, 4152−4161.
(8) Bills, G. F. In *Endophytic Fungi in Grasses and Woody Plants*; Redlin, S. C., Carris, L. M., Eds.; The American Phytopathological Society Press: St. Paul, MN, 1996; pp 31−65.
(9) (a) Schumacher, R. J.; Hurst, R.; Sullivan, W. P.; McMahon, N. J.; Toft, D. O.; Matts, R. L. *J. Biol. Chem.* **1994**, *269*, 9493−9499. (b) Schulte, T. W.; Blagosklonny, M. V.; Romanova, L.; Mushinski, J. F.; Monia, B. P.; Johnston, J. F.; Nguyen, P.; Trepel, J.; Neckers, L. M. *Mol. Cell Biol.* **1996**, *16*, 5839−5845.
(10) Whitesell, L.; Mimnaugh, E. G.; De Costa, B.; Myers, C. E.; Neckers, L. M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 8324−8328.
(11) Rubinstein, L. V.; Shoemaker, R. H.; Paul, K. D.; Simon, R. M.; Tosini, S.; Skehan, P.; Scudiero, D. A.; Monks, A.; Boyd, M. R. *J. Nat. Cancer Inst.* **1990**, *82*, 1113−1118.
(12) (a) Mirrington, R. N.; Ritchie, E.; Shoppee, C. W.; Sternhell, S.; Taylor, W. C. *Aust. J. Chem.* **1966**, *19*, 1265−1284. (b) Evans, G.; White, N. H. *Trans. Br. Mycol. Soc.* **1966**, *49*, 563−576. (c) Nozawa, K.; Nakajima, S. *J. Nat. Prod.* **1979**, *42*, 374−377. (d) Arai, M.; Yamamoto, K.; Namatame, I.; Tomoda, H.; Omura, S. *J. Antibiot.* **2003**, *56*, 526−532. (e) Hellwig, V.; Mayer-Bartschmid, A.; Muller, H.; Greif, G.; Kleymann, G.; Zitzmann, W.; Tichy, H.-V.; Stadler, M. *J. Nat. Prod.* **2003**, *66*, 829−837.
(13) Wijeratne, E. M. K.; Carbonezi, C. A.; Takahashi, J. A.; Seliga, C. J.; Turbyville, T. J.; Pierson, E. E.; Pierson, L. S., III; VanEtten, H. D.; Whitesell, L.; Bolzani, V. da S.; Gunatilaka, A. A. L. *J. Antibiot.* **2004**, *75*, 541−546.
(14) (a) Ayer, W. A.; Lee, S. P.; Tsuneda, A.; Hiratsuka, Y. *Can. J. Microbiol.* **1980**, *26*, 766−773. (b) Ayer, W. A.; Pena-Rodriguez, L. *Phytochemistry* **1987**, *26*, 1353−1355.
(15) Kwon, H. J.; Yoshida, M.; Nagaoka, R.; Obinata, T.; Beppu, T.; Horinouti, S. *Oncogene* **1997**, *15*, 2625−2631.
(16) (a) Soga, S.; Neckers, L. M.; Schulte, T. W.; Shiotsu, Y.; Akasaka, K.; Narumi, H.; Agatsuma, T.; Ikuina, Y.; Murakata, C.; Tamaoki, T.; Akinaga, S. *Cancer Res.* **1999**, *59*, 2931−2938. (b) Soga, S.; Shiotsu, Y.; Akinaga, S.; Sharma, S. V. *Curr. Cancer Drug Targets* **2003**, *3*, 359−369.
(17) Yang, Z.-Q.; Geng, X.; Solit, D.; Pratilas, C. A.; Rosen, N.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 7881−7889.
(18) Moulin, E.; Zoete, V.; Barluenga, S.; Karplus, M.; Winssinger, N. *J. Am. Chem. Soc.* **2005**, *127*, 6999−7004.
(19) Gunatilaka, A. A. L.; Kingston, D. G. I. In *Studies in Natural Products Chemistry, Vol. 20, Structure and Chemistry (Part F)*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 1998; pp 457−505.
(20) (a) Garbaccio, R. M.; Danishefsky, S. J. *Org. Lett.* **2000**, *2*, 3127−3129. (b) Garbaccio, R. M.; Stachel, S. J.; Baeschlin, D. K.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 10903−10908.
(21) Roe, S. M.; Prodromou, C.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *J. Med. Chem.* **1999**, *42*, 260−266.
(22) (a) Pratt, W. B. *Proc. Soc. Exp. Biol. Med.* **1998**, *217*, 420−434. (b) Bagatell, R.; Beliakoff, J.; David, C. L.; Marron, M. T.; Whitesell, L. *Int. J. Cancer* **2005**, *113*, 179−188.
(23) Schulte, T. W.; An, W. G.; Neckers, L. M. *Biochem. Biophys. Res. Commun.* **1997**, *239*, 655−659.
(24) Zou, J.; Guo, Y.; Guettouche, T.; Smith, D. F.; Voellmy, R. *Cell* **1998**, *94*, 471−480.
(25) Grenert, J. P.; Sullivan, W. P.; Fadden, P.; Haystead, T. A.; Clark, J.; Minaugh, E.; Krutzsch, H.; Ochel, H. J.; Schulte, T. W.; Sausville, E.; Neckers, L. M.; Toft, D. O. *J. Biol. Chem.* **1997**, *272*, 23843−23850.
(26) Turbyville, T. J.; Wijeratne, E. M. K.; Whitesell, L.; Gunatilaka, A. A. L. *Mol. Cancer Ther.* **2005**, *4*, 1569−1576.
(27) Fitzpatrick, D. W.; Picken, C. A.; Murphy, L. C.; Buhr, M. M. *Comp. Biochem. Physiol. C* **1989**, *94*, 691−694.
(28) Minervini, F.; Giannoccaro, A.; Cavallini, A.; Visconti, A. *Toxicol. Lett.* **2005**, *159*, 272−283.

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