Derrubone, an Inhibitor of the Hsp90 Protein Folding Machinery

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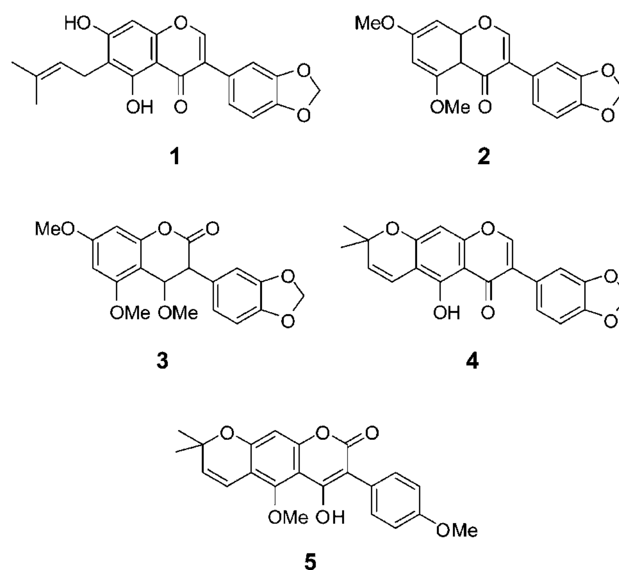
Received April 25, 2007

High-throughput screening of a library of diverse molecules has identified derrubone (**1**), an isoflavone natural product from *Derris robusta*, as a potent Hsp90 inhibitor. Subsequent testing in several cellular-based assays established **1** as a low micromolar inhibitor in vitro. In addition, derrubone induced the degradation of numerous Hsp90 client proteins, a hallmark effect resulting from Hsp90 inhibition. The identification of **1** as an Hsp90 inhibitor provides a new natural product scaffold upon which the development of novel Hsp90 inhibitors can be pursued.

Inhibition of the 90 kDa family of heat shock proteins (Hsp90) has become a therapeutic strategy for numerous diseases because of its well-defined role as a molecular chaperone. Hsp90 is responsible for the conformational maturation of nascent polypeptides and the refolding of denatured proteins into biologically active tertiary and quaternary structures.[1] Client proteins dependent upon Hsp90 have been implicated in all six hallmarks of cancer, and inhibitors of Hsp90 provide a combinatorial attack on multiple signaling pathways responsible for malignant cell growth.[2] Currently, there are more than 20 clinical trials in progress for the treatment of several cancers with derivatives of the natural product Hsp90 inhibitor, geldanamycin (GA).[3] In addition, recent work has demonstrated that Hsp90 is a potential therapeutic target for various neurodegenerative disorders in which aggregated proteins are a defining pathology, including Alzheimer's[4,5] and Parkinson's disease.[6] The potential of Hsp90 as a therapeutic target for numerous disease states has made the identification and optimization of novel Hsp90 inhibitors an emerging therapeutic strategy.

To date, three natural products have been identified as Hsp90 inhibitors. GA and radicicol bind Hsp90 at the N-terminal ATP binding site and prevent Hsp90-catalyzed ATP hydrolysis, dimerization of the N-terminal domains, and subsequent "clamping" of Hsp90 around the bound client protein–substrate.[7] Radicicol has no inhibitory activity in vivo due to the electrophilic nature of the $\alpha,\beta,\gamma,\delta$-unsaturated carbonyl and the allylic epoxide.[8] In contrast, GA is highly active in vivo, and three derivatives, 17-AAG, 17-DMAG, and 17-AAG hydroquinone, are currently being evaluated in clinical trials.[3] Novobiocin, a coumarin antibiotic, was recently shown to bind a previously unrecognized C-terminal nucleotide-binding site on Hsp90.[9] Unfortunately, it demonstrates weak inhibitory activity in cellular-based assays (IC_{50} ca. 500–700 μM) compared to the N-terminal inhibitors (IC_{50} < 100 nM).[10,11] Two related coumarin antibiotics, chlorobiocin and coumermycin A1, also bind to the C-terminus of Hsp90 and are approximately 2–10-fold more active than novobiocin, respectively.[10,11]

In an effort to identify new Hsp90 inhibitors, several groups have developed high-throughput screening (HTS) assays and used these methods to identify new scaffolds that inhibit the Hsp90 protein folding machinery. Recently, we reported the optimization of a rabbit reticulocyte lysate (RRL)-based assay for HTS and utilized this format to screen a library of diverse molecules for Hsp90 inhibition.[12] In this assay, the renaturation of thermally denatured firefly luciferase is dependent upon Hsp90, and the presence of an Hsp90 inhibitor prohibits the refolding of luciferase, which significantly reduces the bioluminescence of the luciferin product. GA[13] and novobiocin[12] display Hsp90 inhibitory activity in the RRL assay, demonstrating that both N- and C-terminal inhibitors can be detected via this approach. Upon screening a large library of known drugs, experimental bioactives, and pure natural products, we identified the natural product derrubone (**1**) as a new Hsp90 inhibitor.



Derrubone (**1**) was originally isolated and characterized as one of a series of structurally related isoflavonoids derived from the Indian tree *Derris robusta*.[14] Isolation from this source provided four isoflavones (including **1**) and five 3-aryl-4-hydroxycoumarins related to **1**. However, to date, no biological activity for these compounds has been reported. Soon after its discovery, the synthesis of **1** was reported, but no additional studies were undertaken.[15] During our HTS for natural product inhibitors of Hsp90, we identified **1** as a "hit" based on the inhibition of luciferase refolding[12] at a concentration of 20 μM. Control experiments demonstrated that derrubone did not inhibit the enzymatic activity of native luciferase, and the activity of luciferase was maintained upon preincubation of the native enzyme with derrubone in reticulocyte lysate (data not shown). Subsequent studies were performed at various concentrations, and **1** demonstrated potent Hsp90 inhibitory activity with an IC_{50} value of 0.23 ± 0.04 μM (Figure 1A). To our surprise, all of the related isoflavones and hydroxycoumarins isolated from the same source, including der-

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10.1021/np070190s CCC: $37.00 © 2007 American Chemical Society and American Society of Pharmacognosy
Published on Web 11/17/2007

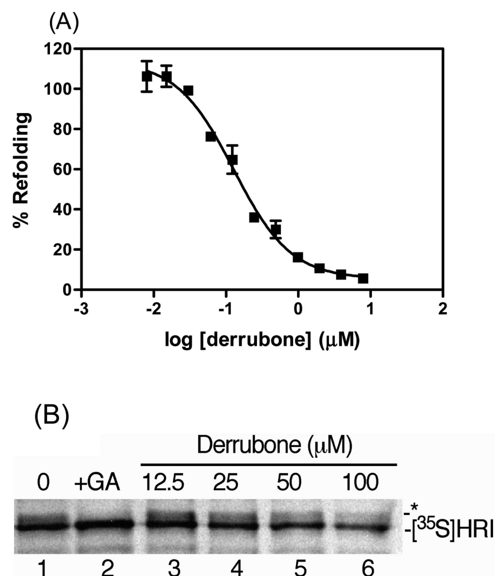

Figure 1. Derrubone (**1**) inhibition of Hsp90. (A) Inhibition of firefly luciferase refolding. Data points correspond to one representative experiment performed in triplicate. (B) Inhibition of HRI maturation and activation by derrubone. His-tagged HRI was synthesized by coupled transcription−translation in reticulocyte lysate in the presence of [^{35}S]methionine. After 10 min of synthesis, derrubone at the indicated concentrations (lanes 3–6), geldanamycin (10 μM, lane 2), or an equivalent volume of DMSO (0, lane 1) was added and synthesis was continued for an additional 30 min. Subsequently, samples were diluted into 7 volumes of heme-deficient lysate and incubated for an additional 40 min. The His-tagged HRI was adsorbed from samples by the addition of NTA-Ni resin on ice for 1 h. Resins were washed, eluted by boiling in SDS-sample buffer, and samples were analyzed by SDS-PAGE and autoradiography after electrotransfer to PVDF membrane (*, activated (autophosphorylated) HRI).

rustone (**2**), derrusnin (**3**), robustone (**4**), and robustic acid (**5**), exhibited no Hsp90 inhibitory activity.

To investigate the mechanism by which derrubone inhibited the Hsp90-dependent refolding of luciferase, denatured luciferase was incubated in reticulocyte lysate in the presence or absence of 100 μM derrubone for 20 min followed by immunoadsorption of Hsp90 or luciferase. Western blotting of these samples indicated that derrubone had no effect on the amount of luciferase that was coadsorbed with Hsp90 (data not shown). Similarly, equivalent amounts of Hsp90 were coadsorbed with luciferase in the presence or absence of derrubone (data not shown). In addition, the complexes formed between Hsp90 and luciferase in the presence of **1** were stable to washing with buffer containing 0.5 M NaCl. These results indicate that derrubone does not inhibit luciferase refolding by generating some component that competes with luciferase for Hsp90 binding nor does it act to sequester denatured luciferase from Hsp90. Thus, **1** does not act by blocking the interaction of Hsp90 with denatured luciferase, but rather it appears that it could possibly act in a manner analogous to molybdate, by stabilizing Hsp90−client interactions and preventing the progression of Hsp90/cochaperone complex containing bound client through its reaction cycle.

To further verify the effect of **1** on the Hsp90 chaperone machine, its ability to inhibit the Hsp90-dependent maturation and activation of the heme-regulated eIF2α kinase (HRI) in heme-deficient reticulocyte lysate was examined. Derrubone inhibited HRI maturation and activation in a concentration-dependent manner (Figure 1B), as evidenced by a decrease in the amount of HRI exhibiting slower electrophoretic mobility indicative of HRI autophosphorylation.[16,17] However, a higher concentration of **1** than expected (100 μM) was required to inhibit HRI maturation and activation compared to the inhibition of HRI activation in the presence of 10 μM GA.

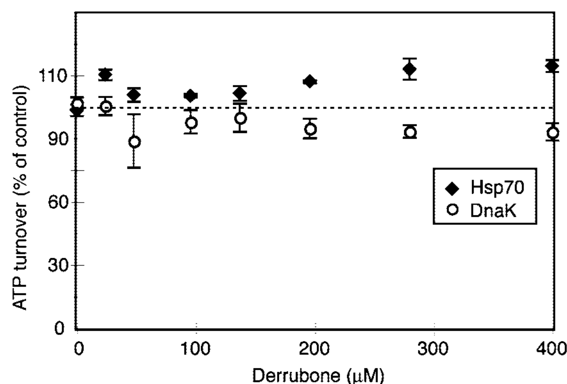

Figure 2. Derrubone does not inhibit the ATPase activity of Hsp70. Compound **1** was added to purified mammalian Hsp70 (1 μM) or bacterial DnaK (1 μM). The intrinsic ATPase activity was measured using malachite green, and the results are plotted as a percentage of the solvent control (DMSO; dotted line). The results are the average of triplicates, and the error bars are the standard deviations. For reference, typical Hsp70 inhibitors reduce the signal by at least 50% (see Experimental Section). Even at high concentrations (up to 400 μM), **1** failed to significantly impact Hsp70's ATPase activity.

Derrubone's ability to inhibit Hsp90 and not Hsp70, which coassemble to form a competent heteroprotein complex with luciferase in reticulocyte lysate, was determined using purified recombinant Hsp70. Incubation of **1** with either mammalian Hsp70 or the bacterial homologue DnaK (Figure 2) resulted in no inhibition of their inherent ATPase activities at up to 400 μM. These results suggest that **1** manifests its activity solely through an interaction with Hsp90.

Proteolytic fingerprinting experiments were carried out to characterize the mechanism by which **1** inhibited luciferase renaturation catalyzed by the Hsp90 chaperone machine. The trypsin fingerprint of Hsp90 in reticulocyte lysate containing up to 500 μM **1** was indistinguishable from the control default Hsp90 fingerprint (DMSO drug vehicle, data not shown). Order of addition experiments indicated that **1** had no effect on trypsin fingerprints of Hsp90 generated from reticulocyte lysate containing geldanamycin, molybdate, or novobiocin, whether derrubone was added before or after the other agents. In addition, **1** (500 μM) did not protect purified Hsp90 C-terminal domain from digestion with trypsin. Furthermore, coimmunoadsorption pull-down assays indicated that derrubone had no discernible effect on the interactions of p23, Cdc37, FKBP52, HOP, or Hsp70 with Hsp90. Thus, like a recent report on the Hsp90 inhibitory activity of celastrol,[18] derrubone appears to inhibit the activity of the Hsp90 chaperone machine by a mechanism that is distinct from GA and novobiocin. Furthermore, these results suggest the possibility that **1** may not interact with Hsp90 present in the cryptic complexes it forms with cochaperones in the absence of interacting client targets.

To test the hypothesis that **1** may inhibit Hsp90 function by stabilizing Hsp90−client interactions and preventing Hsp90s progression through its reaction cycle, we tested the ability of **1** to inhibit geldanamycin-induced inhibition of the interaction of Hsp90 and its cochaperone Cdc37 with the Hsp90-dependent kinase, HRI.[16,19] His-tagged HRI was synthesized in the presence of DMSO, geldanamycin, or **1** for 30 min. DMSO, geldanamycin, or **1** was then added, and their effect on the binding of Hsp90 and Cdc37 to newly synthesized HRI was then assessed by immunoprecipitation of His-tagged HRI (Figure 3). As previously demonstrated,[16] geldanamycin completely blocked the interaction of Cdc37 with HRI, while reducing Hsp90s binding by approximately 80%.

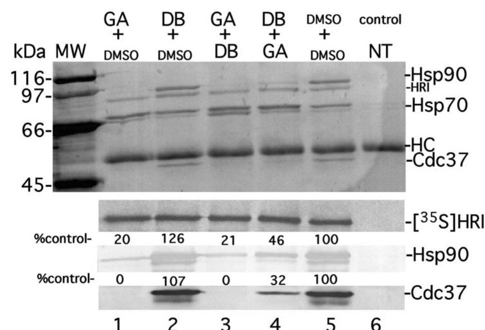

Figure 3. Effect of derrubone (**1**) on geldanamycin-induced dissociation of Hsp90 and Cdc37 from HRI. His-tagged HRI was synthesized by coupled transcription−translation in reticulocyte lysate as described in the Experimental Section. After 10 min of synthesis, geldanamycin (lanes 1 and 3), derrubone (**1**) (lanes 2 and 4), or DMSO (lane 5) was added to the samples, and synthesis was continued for 30 min. DMSO (lanes 1, 2, and 5), derrubone (lane 3), or geldanamycin (lane 4) was then added, and samples were incubated for an additional 10 min. The His-tagged HRI was adsorbed from samples and analyzed for the coadsorption of Cdc37 and Hsp90 as described in the Experimental Section. A sample containing no template (NT) encoding HRI was used as the control for nonspecific binding (lane 6). Upper panel: PVDF membrane stained with Coomassie Blue; MW, molecular weight (kDa) of protein standards; HC, heacy chain of the IgG. Second panel: autoradiogram of immunoprecipitated [^{35}S]labeled HRI. Third panel: Western blot of coadborbed Hsp90. Fourth panel: Western blot of coadsorbed Cdc37. Numbers above the lanes indicate band density relative to that of the control (% control).

The presence **1** had no effect on the interaction of Hsp90 and Cdc37 with HRI, and the addition of **1** after incubation of HRI with geldanamycin had no effect on the geldanamycin-induced dissociation of Hsp90 and Cdc37 from HRI (Figure 3). However, if the reaction mixture was incubated in the presence of **1** prior to the addition of geldanamycin, the binding of Hsp90 and Cdc37 was maintained at 46% and 32% that of the binding observed in the control samples, respectively. Thus, **1** must interact directly with complexes of Hsp90/Cdc37 containing bound HRI, as the presence of **1** antagonized the ability of the Hsp90-specific inhibitor geldanamycin to dissociate Hsp90 and Cdc37 from these complexes. We have previously demonstrated that preincubation of reticulocyte lysate with molybdate similarly inhibits the ability Hsp90 to bind geldanamycin.[20]

Upon verification of the ability of **1** to interact with Hsp90 chaperone complexes, we sought to evaluate the activity of **1** in several cell-based assays. The antiproliferative activity (Figure 4A) of **1** was determined against two distinct human breast cancer cell lines: MCF-7, an estrogen receptor alpha positive (ERα+) cell line, and SkBr3, an ERα- and Her2-overexpressing cell line. The IC_{50} values obtained for antiproliferative activity against MCF-7 and SkBr3 cells were 9 ± 0.7 and 12 ± 0.3 μM, respectively, indicating that **1** is capable of diffusing through the cell membrane and targeting Hsp90 within cells.

A hallmark of Hsp90 inhibition is the ability to induce the degradation of Hsp90-dependent client proteins. Because Hsp90 inhibition prevents the maturation of client protein–substrates through the formation of a destabilized complex, Hsp90-dependent proteins become substrates for the ubiquitin−proteasome pathway, thus abolishing the protein.[21] One such client protein–substrate, the human epidermal receptor 2 (Her2), is a cell-surface tyrosine kinase that mediates signal transduction pathways responsible for cell growth and proliferation.[22] Her2 is overexpressed in 20–40% of human breast cancers and is the target of the clinically used monoclonal antibody herceptin.[23] Derrubone was tested in an ELISA assay using SkBr3 cells to confirm its ability to induce Her2

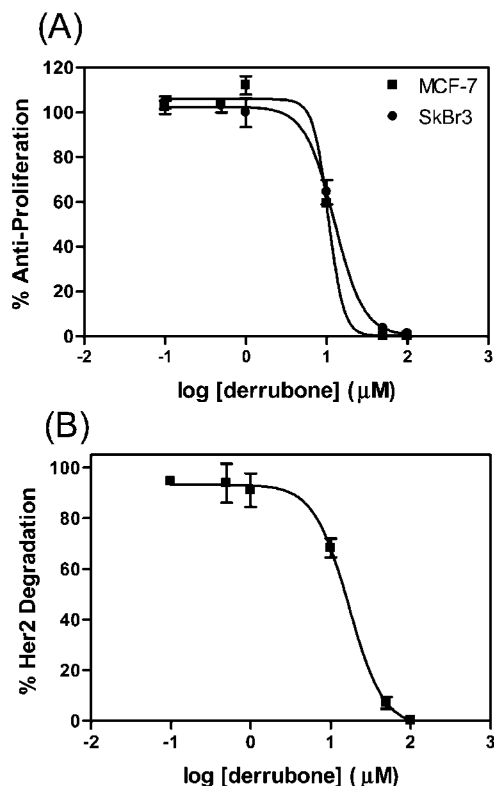

Figure 4. Hsp90 inhibitory activity of derrubone (**1**) in various cell-based assays. (A) Antiproliferative activity of derrubone in two human breast carcinomas. (B) Hsp90 client protein degradation (Her2) in SkBr3 cells. Methods are as described in the Experimental Section.

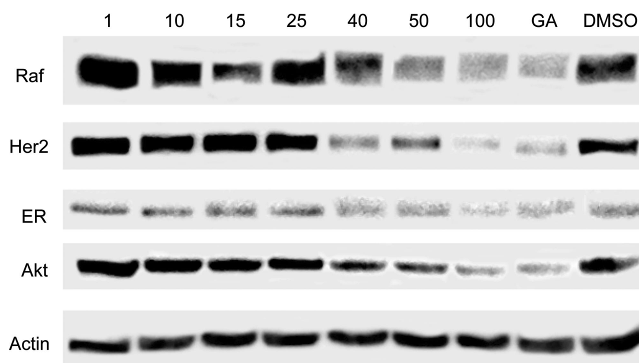

Figure 5. Induced degradation of Hsp90 client proteins via derrubone (**1**) inhibition of Hsp90. Derrubone, at varying concentrations (μM), was evaluated for its ability to downregulate several client proteins as described in the Experimental Section. GA (500 nM) and DMSO were used as positive and negative controls, respectively.

degradation in vitro (Figure 4B). Its activity in this assay ($IC_{50} = 14.0 \pm 1.6$ μM) correlated well to its antiproliferative activity in this cell line, providing a direct link between Her2 degradation and tumor cell viability. The ability of **1** to degrade numerous Hsp90 client proteins, including Raf-1, Akt, and ERα, was also determined in MCF-7 cells via Western blot analysis, as shown in Figure 5. Derrubone induced the degradation of these Hsp90 client proteins in a concentration-dependent manner. Abolishment of the ERα in the MCF-7 cell line suggests that **1** or more potent derivatives may have the potential to treat ERα+ breast carcinomas, a commonality of ~70% of all human breast cancers.[24]

Accordingly, derrubone (**1**) has been identified as a natural product inhibitor of the Hsp90 protein folding machinery. It inhibits

Hsp90-dependent refolding of luciferase and antagonizes the ability of the Hsp90-specific inhibitor geldanamycin to disrupt complexes formed between Hsp90, Cdc37, and their client kinase, HRI. Furthermore, it exhibits potent antiproliferation and Her2 degradation in human breast cancer cell lines. In addition, **1** specifically downregulates numerous Hsp90 client proteins in a concentration-dependent manner. The identification of **1** as an Hsp90 inhibitor provides a new natural product scaffold upon which the development of improved Hsp90 inhibitors can be pursued.

Experimental Section

Luciferase Refolding Assay. To characterize the effect of derrubone on chaperone-dependent refolding of luciferase in reticulocyte lysate, the time course of luciferase renaturation was carried out as described previously.[12] To confirm that the rate of luciferase renaturation was linear over the time period of the assay in the presence of DMSO or the specified concentration of derrubone, samples were taken over the time course of the experiment for quantification of luciferase levels by Western blotting [antiluciferase monoclonal antibody L2164, Sigma (1:1000)]. No breakdown of luciferase was detected in the presence or absence of derrubone over the time course of the experiment.

Hsp90-dependent refolding of firefly luciferase in rabbit reticulocyte lysate[13,25] was then carried out to determine the IC_{50} for inhibition of luciferase refolding by derrubone with the minor modifications to the assay as described previously.[12] Reactions were carried out in triplicate at room temperature in 96-well microtiter plates, and experiments were repeated at least twice, with relative light unit (RLU) production measured using a L_{Max}II (Molecular Devices) microplate reader and a 10 s integration time. The IC_{50} value was determined as the concentration required to inhibit recovery of luciferase activity by 50% relative to the DMSO control. The effect of derrubone on the activity of native luciferase was also assayed as a control as described previously.[12,25] In addition native luciferase was preincubated in reticulocyte lysate under protein synthesis conditions[12,25] in the presence or absence of 100 μM derrubone for 20 min. Derrubone was observed to have no inhibitory effect on the activity of native luciferase and did not affect the stability of the native enzyme incubated in reticulocyte lysate.

Characterization of Derrubone (1) on the Hsp90 Chaperone Machine in Reticulocyte Lysate. Trypsin fingerprinting of Hsp90 containing DMSO (vehicle control), 100 μM derrubone, 10 μM GA, 20 mM molybdate, or 15 mM novobiocin, either alone or in combination, was carried out as previously described.[26] The effect of derrubone (100 μM) on the interaction of p23, Cdc37, FKBP52, HOP, and Hsp70 with Hsp90 in reticulocyte lysate and on Hsp90-dependent maturation of HRI in heme-deficient lysate was also carried out as described previously.[26]

To determine the effect of derrubone on the interaction of Hsp90 with denatured luciferase, denatured luciferase was incubated in reticulocyte lysate for 20 min in the presence of DMSO or 100 μM derrubone under the conditions described above for the determination of derrubone's IC_{50} value. Hsp90 or luciferase was then immunoadsorbed from 100 μL of reaction mixes with anti-Hsp90 (20 μL of OSU anti-Hsp90 prebound to goat antimouse agarose) or antiluciferase (40 μg, Chemicon goat antiluciferase, AB3256 prebound to donkey anti-goat agarose), respectively, as described previously.[26] Immune resins were then washed at both low [10 mM PIPES (pH = 7.2) plus 100 mM NaCl (P100)] or high stringency [10 mM PIPES (pH = 7.2) plus 500 mM NaCl (P500))].[25] Samples were analyzed by SDS-PAGE and Western blotting to quantify coadsorption of luciferase and Hsp90 with one another.

To determine the effect of derrubone on geldanamycin-induced dissociation of Hsp90 and Cdc37 from HRI, His-tagged HRI was synthesized by coupled transcription/translation in reticulocyte lysate in the presence of [^{35}S]methionine as previously described.[27] After 10 min of incubation, geldanamycin (20 μM), derrubone (100 μM), or an equivalent volume of DMSO was added, followed by an additional 30 min of incubation. Subsequently, geldanamycin (20 μM), derrubone (100 μM), or an equivalent volume of DMSO was added, and the reaction mixtures were incubated for an additional 10 min. A reaction mixture lacking plasmid encoding His-tagged HRI was used as a control for nonspecific binding. His-tagged HRI was immunoadsorbed by immobilized antipenta-His antibodies (Qiagen), followed by washing of immunoresins 1× with P100, 2× with P500, and 1× with P50. Samples were separated by SDS-polyacrylamide gel electrophoresis and electrotransferred to a polyvinylidene difluoride (PVDF) membrane. The PVDF membrane was stained with Coomassie Blue, destained, and analyzed by Western blotting for Hsp90 and Cdc37 and autoradiography as previously described.[16,27] Band densities of Hsp90 and Cdc37 on the Western blots were quantified using an AlphaImager HP system and expressed as percent of the band density of the DMSO control.

Purified Hsp70 ATPase Assay. The intrinsic ATP hydrolysis activity of bovine Hsp70 and bacterial DnaK was measured using malachite green, as previously reported.[28] Briefly, 1 μM chaperone was incubated for 3 h at 37 °C with 1 mM ATP and either **1** or a solvent control (DMSO). After this incubation, a malachite green−molybdate solution was added, followed by quenching with 3% sodium citrate. The absorbance was measured at 620 nm in a SpectraMax M5 (Molecular Devices).

Antiproliferative Effects of Derrubone (1). MCF-7 cells were maintained in a 1:1 mixture of Dulbecco's modified Eagle's medium: Ham's F-12 (Gibco) supplemented with nonessential amino acids, L-glutamine (2 mM), streptomycin (500 μg/mL), penicillin (100 units/mL), and 10% fetal bovine serum. SkBr3 cells were maintained in McCoy's 5A media (Iwakata and Grace modification, Cellgro) with L-glutamine supplemented with streptomycin (500 μg/mL), penicillin (100 units/mL), and 10% fetal bovine serum. Cells were grown to confluence in a humidified atmosphere (37 °C, 5% CO_2). The antiproliferative activity of derrubone was determined as described previously.[12] IC_{50} values were calculated using Graphpad Prism from three separate experiments performed in triplicate.

Client Protein Degradation via Her2 ELISA. An ELISA assay to determine the ability of derrubone (**1**) to degrade the Hsp90 client protein Her2 was performed as previously described with minor modifications.[28] Briefly, SkBr3 cells were grown as described above, seeded (3000 cells/well) in 96-well plates, and allowed to attach overnight (37 °C, 5% CO_2). Derrubone or GA, at varying concentrations, was added and the cells were returned to the incubator for 24 h. Media was removed and the cells were washed three times with ice-cold buffer (PBS with 1% Tween). Methanol (−20 °C) was added and the plates were placed at 4 °C for 10 min to permeabilize and fix the cells. The plates were washed again with ice-cold buffer and incubated in blocking buffer (5% BSA in PBST) for 1 h at rt. The plates were incubated with a Her2-specific antibody (rabbit IgG; 1:500 dilution in blocking buffer) at 4 °C overnight. The plates were washed again and incubated at room temperature for 2 h in the presence of an HRP-conjugated antirabbit IgG (1:1000 in blocking buffer). Plates were rinsed, chemiluminescent reagent was added, and the plates were immediately read on a luminometer (Molecular Devices). IC_{50} values were calculated using Graphpad Prism from three separate experiments performed in triplicate.

Western Blot Analysis of Derrubone (1). MCF-7 cells were grown to confluence as described above, seeded in culture dishes (1×10^6/dish), and allowed to attach overnight (37 °C, 5% CO_2). Derrubone was added at varying concentrations and returned to the incubator (37 °C, 5% CO_2) for 36 h. Cells were harvested and analyzed for Hsp90 client protein degradation as described previously.[29]

Acknowledgment. The authors gratefully acknowledge support of this project by the NIH (R01 CA114393) and the Oklahoma Agricultural Experiment Station (Project No. 1975). Lyra Chang provided assistance with the Hsp70 ATPase assays.

References and Notes

(1) Pearl, L. H.; Prodromou, C. *Annu. Rev. Biochem.* **2006**, *75*, 271–294.
(2) Zhang, H.; Burrows, F. *J. Mol. Med.* **2004**, *82*, 488–499.
(3) Pacey, S.; Banerji, U.; Judson, I.; Workman, P. *Handb. Exp. Pharmacol.* **2006**, *172*, 331–358.
(4) Dou, F.; Netzer, W. J.; Tanemura, K.; Li, F.; Hartl, U.; Takashima, A.; Gouras, G. K.; Greengard, P.; Xu, H. *Proc. Natl. Acad. Sci.* **2002**, *100*, 721–726.
(5) Ansar, S.; Burlison, J. A.; Hadden, M. K.; Yu, X. M.; Desino, K. E.; Bean, J.; Neckers, L.; Audus, K. A.; Holzbeierlein, J.; Michaelis, M. L.; Blagg, B. S. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1984–1990.
(6) Shen, H. Y.; He, J. C.; Wang, Y.; Huang, Q. Y.; Chen, J. F. *J. Biol. Chem.* **2005**, *280*, 39962–39969.
(7) Blagg, B. S. J.; Kerr, T. D. *Med. Res. Rev.* **2006**, *26*, 310–338.
(8) Agatsuma, T.; Ogawa, H.; Akasaka, K.; Asai, A.; Yamashita, Y.; Mizukami, T.; Akinaga, S.; Saitoh, Y. *Bioorg. Med. Chem.* **2002**, *10*, 3445–3449.

(9) Marcu, M. G.; Chadli, A.; Bouhouche, I.; Catelli, M.; Neckers, L. M. *J. Biol. Chem.* **2000**, *275*, 37181–37186.
(10) Marcu, M. G.; Schulte, T. W.; Neckers, L. *J. Natl. Cancer Inst.* **2000**, *92*, 242–248.
(11) Burlison, J. A.; Blagg, B. S. J. *Org. Lett.* **2006**, *8*, 4855–4858.
(12) Galam, L.; Hadden, M. K.; Ma, Z.; Ye, Q.-Z.; Yun, B.-G.; Blagg, B. S. J.; Matts, R. L. *Bioorg. Med. Chem.* **2007**, *15*, 1939–1946.
(13) Thulasiraman, V.; Matts, R. L. *Biochemistry* **1996**, *35*, 13443–13450.
(14) East, A. J.; Ollis, W. D.; Wheeler, R. E. *J. Chem. Soc. C: Org.* **1969**, *3*, 365–374.
(15) Jain, A. C.; Jain, S. M. *Tetrahedron* **1972**, *28*, 5063–5067.
(16) Shao, J.; Grammatikakis, N.; Scroggins, B. T.; Uma, S.; Huang, W.; Chen, J.-J.; Hartson, S. D.; Matts, R. L. *J. Biol. Chem.* **2001**, *276*, 206–214.
(17) Shao, J.; Hartson, S. D.; Matts, R. L. *Biochemistry* **2002**, *41*, 6770–6779.
(18) Hieronymus, H.; Lamb, J.; Ross, K. N.; Peng, X. P.; Clement, C.; Rodina, A.; Nieto, M.; Du, J.; Stegmaier, K.; Raj, S. M.; Maloney, K. N.; Clardy, J.; Hahn, W. H.; Chiosis, G.; Golub, T. R. *Cancer Cell* **2006**, *10*, 321–330.
(19) Uma, S.; Hartson, S. D.; Chen, J.-J.; Matts, R. L. *J. Biol. Chem.* **1997**, *272*, 11648–11656.
(20) Hartson, S. D.; Thulasiraman, V.; Huang, W.; Whitesell, L.; Matts, R. L. *Biochemistry* **1999**, *38*, 3837–3849.
(21) Kamal, A.; Thao, L.; Sensintaffar, J.; Zhang, L.; Boehm, M. F.; Fritz, L. C.; Burrows, F. J. *Nature* **2003**, *425*, 407–410.
(22) Slamon, D. J.; Godolphin, W.; Jones, L. A.; Holt, J. A.; Wong, S. G.; Keith, D. E.; Levin, W. J.; Stuart, S. G.; Udove, J.; Ullrich, A. *Science* **1989**, *244*, 707–712.
(23) Plosker, G. L.; Keam, S. J. *Drugs* **2006**, *66*, 449–475.
(24) Ariazi, E. A.; Ariazi, J. L.; Cordera, F.; Jordan, V. C. *Curr. Top. Med. Chem.* **2006**, *6*, 181–202.
(25) Thulasiraman, V.; Matts, R. L. In *Methods in Molecular Biology: Bioluminescent Protocols*; LaRossa, R., Ed.; Humana Press, Inc.: Totowa, NJ, 1997;Chapter 11.
(26) Yun, B.-G.; Huang, W.; Leach, N.; Hartson, S. D.; Matts, R. L. *Biochemistry* **2004**, *43*, 8217–8229.
(27) Prince, T.; Sun, L.; Matts, R. L. *Biochemistry* **2005**, *44*, 15287–15295.
(28) Huezo, H.; Vilenchik, M.; Rosen, N.; Chiosis, G. *Chem. Biol.* **2003**, *10*, 629–634.
(29) Chang, L.; Bertelsen, E. B.; Wisen, S.; Larsen, E. M.; Zuiderweg, E. R.; Gestwicki, J. E. *Biochemistry* **2007**, in press.

NP070190S