Synthesis and Biological Evaluation of a New Class of Geldanamycin Derivatives as Potent Inhibitors of Hsp90

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The heat shock protein Hsp90 has increasingly become an important therapeutic target especially for treatment of cancers. Inhibition of the ATPase activity of Hsp90 by natural products (e.g., 17-allylaminogeldanamycin or radicicol) leads to the ubiquitination of oncogenic client proteins such as Her-2, Raf-1, and p-Akt followed by their proteasomal degradation. Hsp90 inhibitors simultaneously target multiple oncogenic proteins and provide an advantage for cancer therapy due to the potential for increased efficacy and overcoming drug resistance. In an effort to convert geldanamycin into a druglike compound with better pharmacokinetic properties and efficacy in human tumor xenograft models, geldanamycin was derivatized on the 17-position to prepare new analogues such as 17-geldanamycin amides, carbamates, and ureas and 17-arylgeldanamycins. All the compounds were first evaluated ex vivo using a cell-based Her-2 degradation assay and in vitro using biochemical assays that measure recombinant Hsp90 (rHsp90) competitive binding and changes in rHsp90 conformation. In addition, we confirmed the selectivity of geldanamycin analogues for Hsp90 derived from tumor cells using a novel cell lysate binding assay.

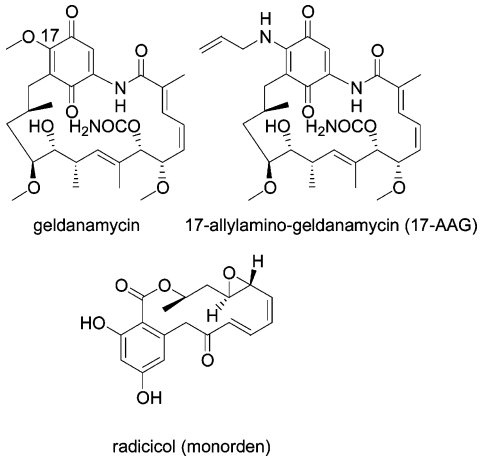
Introduction

Deregulation of signaling proteins in cells by mutation or overexpression results in the disruption of normal cellular function including growth, differentiation, proliferation, and ultimately survival. When key oncogenic proteins are affected, one of the results is the formation of cancer cells and tumor growth. Consequently, selective modulators of signaling proteins such as kinases and nuclear receptors have become important molecular targets for identifying new anticancer agents. This approach has been rewarded by the discovery of several effective modulators of cellular regulatory pathways including Tamoxifen,[1] Casodex,[2] Herceptin,[3] Iressa,[4] and Gleevec.[5]

Hsp90 is an ATP-dependent molecular chaperone that consists of two cytosolic isoforms in human: Hsp90α and Hsp90*β*. In cells, Hsp90 is complexed to an array of cochaperones (e.g., Hop, aha1, cdc37, p23) to form a superchaperone complex that interacts with client oncogenic proteins involved in signal transduction, cell cycle regulation, and apoptosis.[6]

Of great therapeutic interest, selective modulation of Hsp90 is achievable either by inhibiting its ATPase activity by natural products such as geldanamycin and radicicol[7] (Scheme 1) or, as recently described, by inducing its direct degradation via hypericin treatment.[8] Furthermore, inhibition of Hsp90 leads to degradation of multiple key proteins that depend on the interaction with Hsp90 for maintaining their conformation, their stability, and their function. Simultaneously targeting multiple oncogenic proteins provides an advantage for cancer therapy due to the potential for increased efficacy and overcoming drug resistance, which occurs in many cancers including certain forms of leukemia,[9] prostate,[10] and breast cancers.[11] For example, many early stage chronic myelogenous leukemia (CML) patients respond to the new kinase inhibitor Gleevec, but then they become resistant over time. This is often due to mutations in the Bcr-Abl kinase that prevent the drug from binding. Similarly, early stage prostate cancer patients often respond to anti-androgen therapy but develop resistance due to mutations in the androgen receptor. Hsp90 inhibitors selectively cause the degradation of mutant kinases and other mutant client proteins and thus are likely to be effective where conventional inhibitor therapy fails. In the case of the signal transduction protein Her-2/neu, a key driver of breast cancer growth, Hsp90 inhibition leads to its ubiquitination and

Scheme 1



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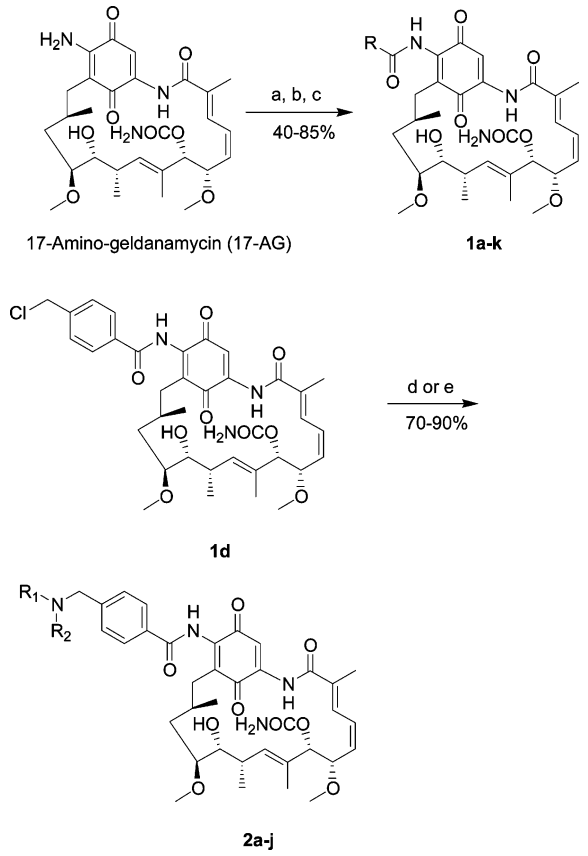
degradation by the proteasome.[12] Similarly, inhibition of Hsp90 induces the degradation of the mutant androgen receptor,[13] a gene transcription factor critical to the growth of both hormone-dependent and hormone-independent prostate cancer. At the same time, Hsp90 inhibitors down-regulate the PI3K/Akt pathway leading to the deactivation[14] or degradation of Akt and subsequently reducing the expression of D-cyclins.[15] This process of deactivation also applies to other client proteins such as tyrosine kinase FLT3[16] involved in signal transduction in leukemia cells. Consequently, the ability of Hsp90 inhibitors to degrade both normal and mutant client proteins provides the opportunity to treat currently incurable late-stage resistant tumors.

In 1994, Whitesell et al.[17] discovered that geldanamycin (Scheme 1), a naturally occurring benzoquinone ansamycin isolated from the culture broth of *Streptomyces hygroscopicus*, bound very tightly to the N-terminal ATPase domain of rHsp90 (K_d = 1.2 μM).[18] Because geldanamycin is too chemically and metabolically unstable to become a drug, it has been derivatized, mainly on the 17-position of the quinone moiety, leading to a plethora of 17-alkylaminogeldanamycins including 17-allylamino-17-demethoxygeldanamycin (17-AAG). This latter is a potent inhibitor of Hsp90, and largely as a result of its excellent in vitro potency, the National Cancer Institute has initiated phase I clinical trials for advanced cancer patients. Although 17-AAG is a very potent Hsp90 inhibitor, it also suffers from pharmaceutic deficiencies including difficult formulation challenges. As a result, we investigated methods to prepare novel analogues of geldanamycin with improved chemical and metabolic stability and increased formulation options such as water solubility while retaining high potency in functional cell-based assays and in vivo models.

Chemistry

Geldanamycin is a member of the ansamycin family of natural products and displays two key structural features, the quinone moiety and the ansa ring, which are responsible, via an induced fit,[19] for its binding to the N-terminal ATPase domain of Hsp90. The goal of our synthetic program was to improve the pharmaceutical properties[20] and chemical stability of geldanamycin by modifying the quinone moiety. Recent studies of the redox potential of mitomycins[21] show that it is possible to tune the electronic properties of the quinone by modifying substituents in various positions of the ring. Because synthetic geldanamycin analogues having amides, carbamates, or carbon−carbon bonds attached directly to the 17-position had never been described, we directed our effort toward their synthesis. Following a reported procedure,[22] geldanamycin was treated with ammonia in methanol to afford 17-amino-17-demethoxygeldanamycin (17-AG). Because of the deactivating effect of the quinone, this latter compound was unreactive toward N-acylation using standard procedures. We were able to circumvent this problem by using a three-step route: (1) reduction of the quinone moiety of 17-AG with 10% sodium thionite, (2) addition of the desired acyl chloride in the presence of molecular sieves, and (3) air oxidation of the dihydroquinone mediated by $CuSO_4$ and Hunig's base (Scheme 2). It is of interest to note that without the presence of base, the oxidation was much slower. This three-step sequence gave rise to the geldanamycin amides **1a**−**k** with moderate to good yields. Treatment of the geldanamycin analogue **1d** with a series of secondary amines under standard conditions gave rise to geldanamycin amides **2a**−**j** (Scheme 2). Replacing the acyl chloride by either an alkyl chloroformate or an alkyl isocyanate in the second step of the sequence gave access to the geldanamycin carbamates **4a**−**d** and the geldanamycin urea **5** with good yields (Scheme 3). For the synthesis of the 17-arylgeldanamycins, we first hydrolyzed geldanamycin and then, using standard conditions, converted the resulting 17-hydroxy-17-demethoxygeldanamycin into the triflate **6**, which is a stable yellow solid (Scheme 4). Several conditions were attempted for the Suzuki coupling, and we obtained the best results using Neel's conditions,[23] which afforded the 17-arylgeldanamycins **7a**,**b** in moderate yields.

Scheme 2[a]

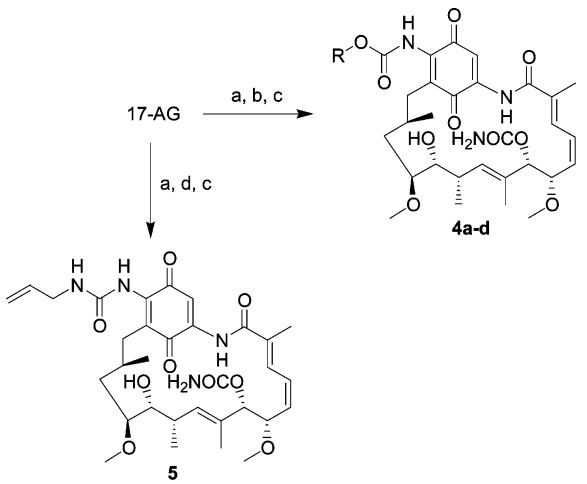


[a] (a)$Na_2S_2O_4$ 10%, AcOEt' (b) RCOCl, THF, 4 Å molecular sieves, 0 °C to room temp; (c) $CuSO_4$, MeOH, EtN(*i*-Pr)$_2$; (d) Me_2NH, HCl salt, EtN(*i*-Pr)$_2$, CH_2Cl_2, reflux; (e) R_1R_2NH, THF, NaI, reflux.

Biological Evaluation

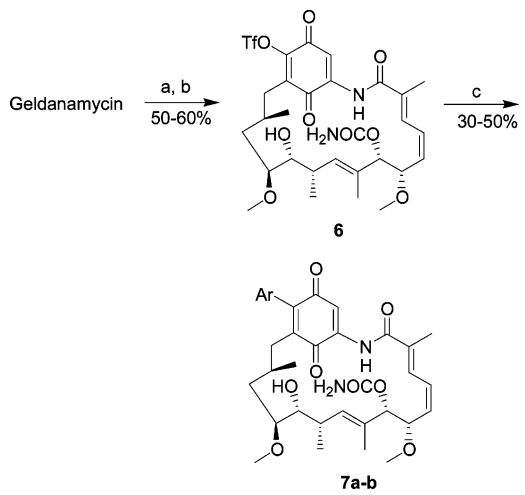
The in vitro biological activity of the new geldanamycin analogues was evaluated using a human epidermal growth factor 2 (Her-2) degradation assay, a competitive rHsp90 binding assay, a cell lysate binding assay, a bis-ANS conformational assay, and a cytotoxic assay (MTS). The Her-2 degradation assay measures the degradation of Her-2 in MCF7 tumor cells resulting from inhibition of the ATPase activity of Hsp90. The competitive binding assays utilized the biotinylated

Scheme 3[a]



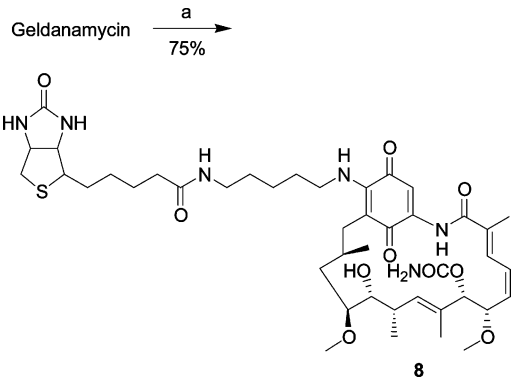
[a] (a) $Na_2S_2O_4$, 10%, AcOEt; (b) ROCOCl, THF, 4 Å molecular sieves, 0 °C to room temp; (c) $CuSO_4$, MeOH, $EtN(i\text{-}Pr)_2$; (d) allyl isocyanate, THF, 0 °C to room temp.

Scheme 4[a]



[a] (a) $Ba(OH)_2$, THF/H_2O, 65 °C; (b) Tf_2O, $(i\text{-}Pr)_2NEt$, CH_2Cl_2; (c) $ArB(OH)_2$, Pd_2dba_3, CsBr, CsF, dioxane.

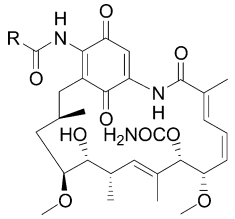
Scheme 5[a]



[a] (a) 5-(Biotinamido)pentylamine, THF, room temp.

geldanamycin derivative **8**, which was prepared in one step from 5-(biotinamido)pentylamine and geldanamycin (Scheme 5). The 1,1′-bis(4-anilino-5-naphthalenesulfonic acid) (bis-ANS) is an environmentally sensitive fluorophore that becomes fluorescent when it binds and induces a conformational change in rHsp90. This assay has been previously used to look at the conformational changes of Grp94,[24] an endoplasmic reticulum homologue of cytosolic Hsp90. Finally, selected analogues were evaluated in mouse xenograft models of human tumors for pharmacokinetic and efficacy studies.

Table 1. Activity of Geldanamycin Amides



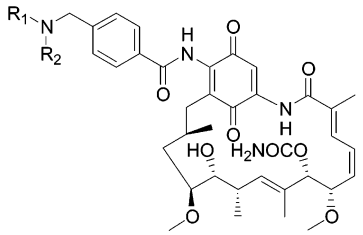
compd	R	Her-2 IC_{50} (nM)[a,b]	rHsp90 IC_{50} (μM)[a,c]	b-ANS rHsp90 IC_{50} (μM)[a,d]
1a	phenyl	200	3	4
1b	2-furyl	500	4	4
1c	2-thienyl	400	3	2
1d	*p*-chloromethyl-phenyl	550	2	4
1e	*m*-chloromethyl-phenyl	1000	10	ND[e]
1f	acetyl	1700	20	15
1g	*o*-anisyl	180	1	1.1
1h	*m*-anisyl	180	0.9	1.3
1i	*p*-anisyl	400	4	5
1j	*p*-nitrophenyl	300	2	3.5
1k	*p*-fluorophenyl	250	1.5	1.5

[a] Values correspond to $n = 2$. [b] Standard errors in the range of 15−20%. [c] Standard errors in the range of 15−20%. [d] Standard errors in the range of 35−40%. [e] ND = not determined. For assay conditions, see Experimental Section.

Results and Discussion

The primary assay for evaluating the structure−activity relationships of new geldanamycin derivatives was the Her-2 degradation assay in intact MCF7 cells using a flow cytometric assay based on fluorescence-activated cell sorter technology (FACS). By use of 17-AAG as a reference (IC_{50} = 15 ± 4 nM)[25] in this functional assay, the screening of the first series of geldanamycin amides revealed a decrease in activities ranging from 180 to 1700 nM (Table 1). Among those amides, aromatic derivatives such as **1a**, **1d**, **1e**, **1j**, and **1k** exhibited better potencies than their aliphatic counterpart **1f**; furthermore, changing the size and the polarity of the aromatic ring from phenyl to furyl and thienyl (**1b**, **1c**) led to a 2-fold drop in activity. A study of the substituent effects showed that an electron-donating group on the ortho or meta position (**1g**, **1h**) was better than on the para position (**1i**). For analogues **1d** and **1e**, the chloromethyl group reverses the position effect with the para isomer being twice as active as the meta isomer. On the other hand, electron-withdrawing groups on the para position (**1j**, **1k**) maintain the activity at 250 nM. To improve the formulation properties of the geldanamycin amides, we prepared a second series of geldanamycin analogues by adding a dialkylaminomethyl group on the para position of the amide **1a**. The addition of an ionizable amino group provided water solubility via the salt form and was more readily formulatable for intravenous administration (free base solubility range, 10−20 μg/mL; salt solubility range, 5−40 mg/mL). As exemplified by compound **2a** (Table 2), addition of a dimethylamino methyl group led to a

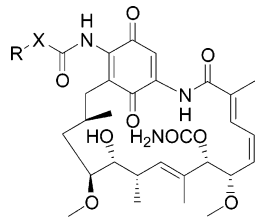
Table 2. Activity of Water-Soluble Geldanamycin Amides



compd	R_1R_2N	Her-2 IC_{50} (nM)[a]	rHsp90 IC_{50} (μM)[a]	b-ANS rHsp90 IC_{50} (μM)[a]
2a	dimethylamino	1700	3.0	17.0
2b	1-(4-(4-chlorophenyl))piperazinyl	250	1.0	1.0
2c	1-(4-(4-methoxyphenyl))piperazinyl	220	1.5	3.0
2d	1-(4-(4-nitrophenyl))piperazinyl	200	1.3	1.0
2e	1-(4-methyl)piperazinyl	800	8.0	5.0
2f	1-(4-cyclohexyl)piperazinyl	600	5.8	5.5
2g	piperidinyl	>10000	2.1	2.5
2h	dipropylamino	500	1.2	3.0
2i	benzylethylamino	140	1.1	1.0
2j	1,2,3,4-tetrahydroisoquinolinyl	200	1.5	1.0

[a] Values correspond to $n = 2$. For assay conditions, see Experimental Section.

Table 3. Activity of Geldanamycin Carbamates and Ureas

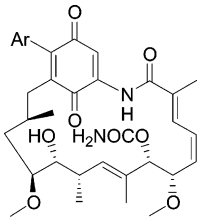


compd	R	X	Her-2 IC_{50} (nM)[a]	rHsp90 IC_{50} (μM)[a]	b-ANS rHsp90 IC_{50} (μM)[a]
4a	ethyl	O	260	3.0	3.0
4b	*p*-acetoxybenzyl	O	50	2.5	1.0
4c	isobutyl	O	120	4.0	2.0
4d	4-(*N*-morpholino)butyl	O	500	1.0	1.0
5	allyl	NH	>10000	ND[b]	35.0

[a] Values correspond to $n = 2$. [b] ND = not determined. For assay conditions, see Experimental Section.

9-fold decrease in activity. The screening of various tertiary amines showed that the compounds with benzylalkylamino groups (**2i**) were three times more active than the analogues with dialkylamino groups (**2h**). Moreover, in the cyclic amine series, the 4-arylpiperazine derivatives **2b**−**d** were much more potent than the piperidine derivative **2g**. In a second SAR study, we examined geldanamycin carbamate derivatives and found their activities to be within the same range of potencies as the amides. Carbamate **4b** (Table 3) was the most active derivative due to release of 17-AG in the cell via hydrolysis followed by 1,6-elimination of the quinone methide moiety.[26] Compound **4d** was 4-fold less active than **4c**, showing a detrimental effect of both the extension of the alkyl chain and the addition of a high pK_a tertiary amine such as a morpholino group. In comparison to the amide series for which 17-alkylamido groups were only weakly active, the alkyl carbamates were very potent. For the aryl carbamates, no data were produced because of their chemical instability that prevented them from being successfully isolated. For reasons that are still unclear, the 17-urea-geldanamycin derivatives were completely inactive in this assay. 17-Arylgeldanamycins **7a**,**b** are approximatively 2-fold less active than the best amide **2i** in the Her-2 degradation assay, indicating that replacing the amide by an aryl group on the 17-postion of geldanamycin affects the activity only moderately (Table 4).

Table 4. Activity of 17-Arylgeldanamycins



compd	Ar	Her-2 IC_{50} (nM)[a]	rHsp90 IC_{50} (μM)[a]	b-ANS rHsp90 IC_{50} (μM)[a]
7a	phenyl	280	3	16.0
7b	2-thienyl	250	1.7	10.0

[a] Values correspond to $n = 2$. For assay conditions, see Experimental Section.

Since the Her-2 degradation assay provides an indirect readout of Hsp90 inhibition, namely, the degradation of Her-2, and indicates that multiple factors are associated with a cell-based assay such as the amount of cell penetration, it was important to confirm the compound bioactivity with biochemical assays that

Table 5. Cell Lysate Hsp90 Binding of 17-AAG, 17-Arylgeldanamycin, and Geldanamycin Amides and Carbamates

compd	cell lysate Hsp90 IC_{50} (nM)[a]	(rHsp90 IC_{50})/ (cell lysate Hsp90 IC_{50})
17-AAG	20	40
1c	40	75
2a	15	200
2b	9	111
2e	120	67
2f	300	19
2i	12	92
4c	3	1333
4d	10	100
7a	70	43
7b	55	31

[a] Values correspond to $n = 2$. Standard errors are in the range of 20−25%. For assay conditions, see Experimental Section.

directly measure their interaction with Hsp90. For this purpose, recombinant Hsp90 (rHsp90) competitive binding and bis-ANS conformational assays were used to further screen our compounds and allowed us to cluster them by relative binding activity. Very active compounds such as **1c**,**g**,**h**,**k** and **2c**,**d**,**i**,**j** bind tightly to rHsp90 and efficiently inhibit the rHsp90 conformational change with IC_{50} values between 1 and 3 μM. In contrast, compounds that are less active or inactive in the Her-2 degradation assay, such as **1b**,**e** and **2a**,**e**, have IC_{50} values between 4 and 20 μM. Nevertheless, the 17-arylgeldanamycins seem to behave differently by performing very poorly in the bis-ANS assay while maintaining good IC_{50} values in the recombinant Hsp90 binding assay. Although the binding and conformational activities tracked with the results of the Her-2 degradation assay, the potency gap between those assays as exemplified for **2i** (Table 2; Her-2, IC_{50} = 140 nM; rHsp90 binding, IC_{50} = 1100 nM; bis-ANS, IC_{50} = 1000 nM) remained to be explained. Also observed for 17-AAG (rHsp90 binding, IC_{50} = 800 nM; bis-ANS, IC_{50} = 700 nM), this discrepancy was recently clarified by showing that the Hsp90 from cell lysates of cancer cells such as MCF7 have 100-fold higher affinity for 17-AAG than does recombinant Hsp90.[27] This increased Hsp90 binding affinity was also observed for our compounds in MCF7 cell lysates (Table 5) with IC_{50} values ranging from 9 to 300 nM for the 17-amide-geldanamycin series, from 3 to 10 nM for the 17-carbamate-geldanamycin series, and from 55 to 70 nM for the 17-arylgeldanamycin series. Before trying to rationalize this affinity increase, we first want to emphasize that the high-affinity Hsp90 in the cell lysate is fully complexed to its cochaperones and that the 100-fold affinity increase occurs only for geldanamycin derivatives and does not occur for other Hsp90 inhibitors such as radicicol (K_d = 19 nM[18]). As shown in a recent study,[28] geldanamycin requires major conformational changes such as isomerization of the amide and rotation of the quinone ring in order to adopt the optimal C-shape conformation.[29] The presence of cochaperones complexed to Hsp90 may facilitate the conformational changes of geldanamycin from the extended to the C-shape conformation by optimizing the interactions between Hsp90 and geldanamycin at each step of this enthropically disfavored process.

Compounds were also screened in the MTS assay in order to determine their cytotoxicity to cancer cells

Table 6. MTS Assay of Geldanamycin Amides and Carbamates

compd	MCF7 cells IC_{50} (μM)[a]
2a	>30
2b	0.15
2f	>30
2h	0.5
2i	0.2
4b	0.04
4c	0.18

[a] Values correspond to $n = 2$. Standard errors are in the range of 40−45%. For assay conditions, see Experimental Section.

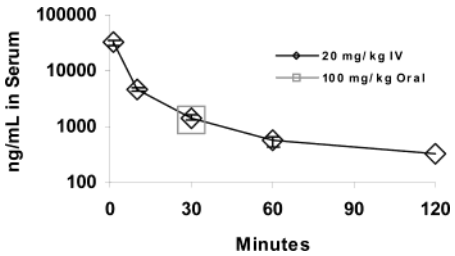


Figure 1. Serum concentrations of **2i** following iv and oral administration to balb/c mice.

(Table 6). For the most potent derivatives such as **2b**, **2h**, **2i**, **4b**, and **4c**, there is a good correlation between their activity in the Her-2 degradation assay (Tables 2 and 3) and in the MTS assay (Table 6). In contrast, the weaker compounds such as **2a** and **2f** do not induce sufficient degradation of key clients such as Her-2 to be cytotoxic in MCF7 cells.

Because one of our goals was to identify geldanamycin derivatives with improved pharmaceutic properties, we evaluated several analogues in vivo. The geldanamycin analogue **2i** was one of the most potent in the in vitro assays, and its pharmacokinetic properties were determined in mice. Following iv administration (Figure 1), **2i** exhibited a high total clearance (79 (mL/min)/kg), a large steady-state volume of distribution (1878 mL/kg), and a terminal half-life of 37 min. These pharmacokinetic parameters are similar to those reported for 17-AAG (microdispersion) administered intravenously to mice.[30] After oral administration, **2i** was detectable in two of three animals at the first sample time (0.5 h) and was below the limit of quantitation in all animals thereafter. We anticipated that the relatively high iv clearance rate of **2i** was likely due to metabolism, and thus, low bioavailability was anticipated for this compound. However, significant metabolite levels of **2i** were not observed in mouse serum following either iv or oral routes of administration. Compounds **2i** and **2b** were subsequently tested for antitumor activity in several human tumor xenograft models in mice using ip administration. Compound **2b** was tested against two lung cancers, the small-cell H69 xenograft (Figure 2a) and the non-small-cell A549 xenograft (Figure 2c). Compound **2i** was tested on the glioblastoma U87 xenograft (Figure 2b) and in the A549 xenograft. Each of the tumors used is dependent on one or more Hsp90 client proteins including Raf-1 (A549), IGF-1 receptor (H69), or Akt (U87). As shown in Figure 2, both compounds displayed potent antitumor activity in each model tested. Compounds **2b** and **2i** were well tolerated when given at low doses daily (parts a and b of Figure 2), at intermediate doses three times per week (Figure 2c),

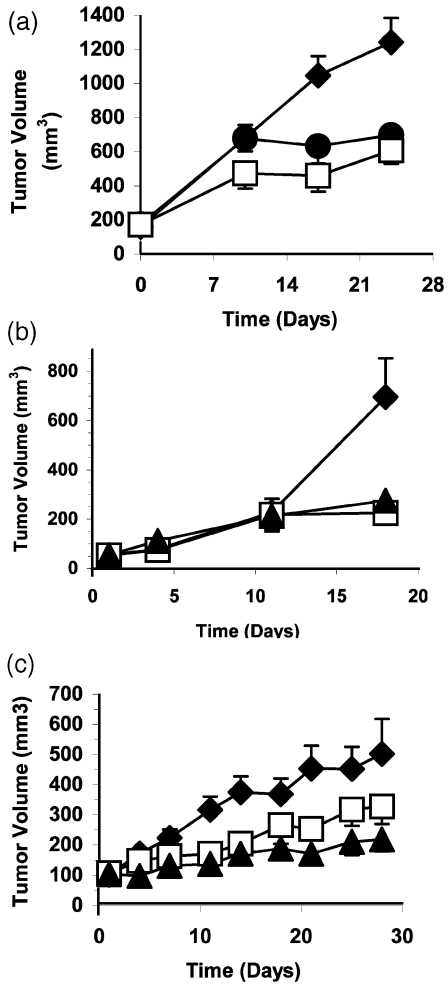


Figure 2. (a) Efficacy of **2b** in H69 small-cell lung cancer: (diamonds) PETA vehicle; (circles) **2b** 20 mg/kg, 7×/week, 4 weeks; (squares) **2b** 30 mg/kg, 7×/week, 4 weeks. All the compounds were administered ip. (b) Efficacy of **2i** in U87 glioblastoma: (diamonds) PETA vehicle; (squares) **2i** 25 mg/kg, 7×/week, 18 days; (circles) **2i** 75 mg/kg, 2×/week, 18 days. All the compounds were administered ip. (c) Efficacy of **2b** and **2i** in A549 lung cancer: (diamonds) PETA vehicle; (squares) **2b** 40 mg/kg, 3×/week, 4 weeks; (circles) **2i** 40 mg/kg, 3×/week, 4 weeks. All the compounds were administered ip.

or at high doses twice weekly (Figure 2b). The lack of schedule dependence along with the pharmacokinetic properties exhibited by both compounds suggest a fast diffusion into the tissues and, particularly, accumulation in solid tumors in mice (data not shown) owing to enhanced retention of ansamycins to the high-affinity form of Hsp90.

Conclusion

During this study, geldanamycin has been derivatized on the 17-position to produce new analogues with common pharmacophores such as amides, ureas, carbamates, and aryl functional groups. After ex vivo optimization of their activity using the Her-2 degradation assay, we were able to refine the data using the bis-ANS and recombinant Hsp90 competitive binding assays. More importantly, it was confirmed that tumor cell lysate Hsp90 has a much higher affinity for this new series of geldanamycin analogues than recombinant Hsp90 does, thus explaining the apparent discrepancy between the binding and the Her-2 degradation assays. This study also demonstrates that converting 17-aminogeldanamycin into the corresponding amide such as **2i** leads to a 10-fold decrease in activity in the Her-2 assay without affecting the cell lysate Hsp90 binding (Table 5), which correlates well with the efficacy data in animal models.

The high selectivity of geldanamycins for tumor cell Hsp90 could confer to this class of compounds a unique status among Hsp90 inhibitors used in oncology. In addition, for other diseases such as viral diseases, autoimmune disorders, and inflammation, for which the cells are highly dependent on Hsp90 for survival, similar selectivity could be observed.

Experimental Section

Chemistry. All reactions were carried out with continuous stirring under an atmosphere of nitrogen. Commercial reagents and solvents were used as received without further purification or drying. ^{1}H NMR were obtained using a Bruker-400 spectrometer. Chemical shifts are reported in parts per million relative to tetramethylsilane as internal standard. Thin-layer chromatography (TLC) was performed on 250 μM silica gel plates (Whatman 4861-820). Flash chromatography was run using EM Science silica gel (230−400 mesh). Low-resolution mass spectra were run using a ThermoFinnigan LCQ spectrometer. High-resolution mass spectra were run at the mass spectrometry facility of UC Riverside using a PE BIOSYSTEMS DE-STR MALDI TOF spectrometer. Melting points were measured using an electrothermal MEL-TEMP apparatus and are reported uncorrected. Compound purity was determined by HPLC analysis using a C18 reverse-phase column with an Agilent 1100 series system attached to a Hewlett-Packard chromatograph manager.

General Procedure for the Synthesis of Geldanamycin Amide Is Exemplified for 17-Benzoylamino-17-demethoxygeldanamycin 1a. A solution of 17-amino-17-demethoxygeldanamycin (3.1 g, 5.62 mmol) in EtOAc (400 mL) was treated with $Na_2S_2O_4$ (10%, 60 mL) at room temperature. After 2 h, the aqueous layer was extracted with EtOAc (100 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to give 18,21-dihydro-17-amino17-demethoxygeldanamycin as a yellow solid. This solid was dissolved in anhydrous THF (70 mL) and transferred via cannula to a mixture of benzoyl chloride (0.65 mL, 5.62 mmol) and 4 Å molecular sieves (4.5 g) in THF (20 mL) at 0 °C. After 12 h at room temperature, EtN(*i*-Pr)$_2$ (1.1 mL), $CuSO_4$ (0.44 g, 2.81 mmol), and MeOH (8 mL) were further added to the reaction mixture. After 3 h, the reaction mixture was filtered and concentrated under reduced pressure. The crude material was dissolved in EtOAc (300 mL) and washed with 2 N HCl (50 mL) and saturated $NaHCO_3$ (50 mL), dried over Na_2SO_4, and concentrated under reduced pressure to give the crude material, which was purified by recrystallization from EtOH to give 17-benzoyl-amino-17-demethoxygeldanamycin as a yellow solid (3.0 g, 82%). R_f = 0.50 in 80:15:5 CH_2Cl_2/EtOAc/MeOH. Mp = 218−220 °C. HPLC purity: 99.9%. ^{1}H NMR $CDCl_3$ δ 0.92−0.94 (m, 6H, C10-Me, C14-Me), 1.70 (br s, 2H, 2xH13), 1.79 (br s, 4H, H14 and C8-Me), 2.03 (s, 3H, C2-Me), 2.56 (dd, 1H, J = 8.7, 13.9 Hz, H15), 2.64 (dd, 1H, J = 4.1, 14.1 Hz, H15), 2.76−2.79 (m, 1H, H10), 3.33 (br s, 7H, 2 × OMe, H11), 3.44−3.46 (m, 1H, H12), 4.325 (d, 1H, J = 9.0 Hz, H6), 5.16 (s, 1H, H7), 5.77 (d, 1H, J = 9.3 Hz, H9), 5.91 (t, 1H, J = 9.9 Hz, H5), 6.57 (t, 1H, J = 11.2 Hz, H4), 6.94 (d, 1H, J = 11.4 Hz, H3), 7.48 (s, 1H, H19), 7.52 (t, 2H, J = 7.4 Hz, Ph), 7.62 (t, 1H, J = 7.2 Hz, Ph), 7.91 (d, 2H, J = 7.3 Hz, Ph), 8.47 (s, 1H, NHCO), 8.77 (s, 1H, N22−H). MS *m*/*z* 672.5 (M^+ + Na). HRMS calculated for $C_{35}H_{43}N_3O_9Na$ (M^+ + Na): 672.2897. Found 672.2903.

General Procedure for the Synthesis of 17-(4-Dialkylaminomethylbenzoyl)-17-amino-17-demethoxygeldanamycin Is Exemplified for 17-(4-Dimethylaminomethylbenzoyl)-17-amino-17-demethoxygeldanamycin 2a. To a

solution of **1d** (3.73 g, 5.3 mmol) in CH_2Cl_2 (50 mL) were added $Me_2NH{\cdot}HCl$ salt (1.51 g, 18.5 mmol), Et(*i*-Pr)$_2$N (2.8 mL), and NaI (0.79 g, 5.3 mmol). The resulting solution was heated at reflux for 6 h, whereupon it was cooled to room temperature, diluted with EtOAc (600 mL), washed with water (100 mL), dried with Na_2SO_4, and concentrated under reduced pressure to give the crude material, which was purified by recrystallization in EtOH to give 17-(4-dimethylaminomethylbenzoyl)-17-amino-17-demethoxygeldanamycin as a yellow solid (1.74 g, 46%). R_f = 0.10 in 80:15:5 CH_2Cl_2/EtOAc/MeOH. Mp = 203−206 °C. HPLC purity: 99.9%. ^{1}H NMR $CDCl_3$ δ 0.93 (d, 3H, J = 6.5 Hz, C10-Me or C14-Me), 0.95 (d, 3H, J = 6.5 Hz, C10-Me or C14-Me), 1.70−1.74 (m, 3H, 2 × H13, H14), 1.79 (s, 3H, C8-Me), 2.04 (s, 3H, C2-Me), 2.29 (s, 6H, N(Me)$_2$), 2.53−2.59 (m, 1H, H15), 2.63−2.67 (m, 1H, H15), 2.75−2.79 (m, 1H, H10), 3.33 (br s, 7H, 2 × OMe, H11), 3.46 (br s, 1H, H12), 3.54 (s, 2H, NC*H*$_2$Ar), 4.33 (d, 1H, J = 9.1 Hz, H6), 5.16 (s, 1H, H7), 5.77 (d, 1H, J = 9.5 Hz, H9), 5.91 (t, 1H, J = 10.0 Hz, H5), 6.57 (t, 1H, J = 11.3 Hz, H4), 6.94 (d, 1H, J = 11.1 Hz, H3), 7.49 (s, 1H, H19), 7.49 (d, 2H, J = 8.0 Hz, Ar), 7.87 (d, 2H, J = 8.2 Hz, Ar), 8.47 (s, 1H, NHCO), 8.77 (s, 1H, N22−H). MS *m*/*z* 707.7 (M^+ + H). HRMS calculated for $C_{38}H_{51}N_4O_9$ (M^+ + H): 707.3656. Found 707.3631.

General Procedure for the Synthesis of Geldanamycin Carbamates Is Exemplified for 17-(Ethoxycarbonyl)-amino-17-demethoxygeldanamycin 4a. A solution of 17-amino-17-demethoxygeldanamycin (0.6 g, 1.10 mmol) in EtOAc (120 mL) was treated with $Na_2S_2O_4$ (10%, 10 mL) at room temperature. After 2 h, the aqueous layer was extracted twice with EtOAc and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to give 18,21-dihydro-17-amino-17-demethoxygeldanamycin as a yellow solid. This solid was dissolved in anhydrous THF (5 mL) and transferred via cannula to a mixture of ethyl chloroformate (0.11 mL, 1.15 mmol) and 4 Å molecular sieves (1.2 g) in THF (3 mL) at 0 °C. After 12 h at room temperature, Et*N*(*i*-Pr)$_2$ (0.38 mL), $CuSO_4$ (60 mg, 0.37 mmol), and MeOH (2 mL) were further added to the reaction mixture. After 2 h, the reaction mixture was filtered and concentrated under reduced pressure. The crude material was dissolved in EtOAc (100 mL) and washed with 2 N HCl (10 mL) and saturated $NaHCO_3$ (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure to give the crude material, which was purified by flash chromatography to give 17-ethoxycarbonyl-17-amino-17-demethoxygeldanamycin as a yellow solid (0.25 g, 40%). R_f = 0.31 in 80:15:5 CH_2Cl_2/EtOAc/MeOH. Mp = 242−245 °C. HPLC purity: 99.9%. ^{1}H NMR $CDCl_3$ δ 0.93 (d, 3H, J = 6.0 Hz, C10-Me or C14-Me), 0.95 (d, 3H, J = 6.9 Hz, C10-Me or C14-Me), 1.32 (t, 3H, J = 7.2 Hz, OCH$_2$-*Me*), 1.62−1.74 (m, 3H, 2 × H13, H14), 1.78 (s, 3H, C8-Me), 2.02 (s, 3H, C2-Me), 2.58 (m, 2H, 2 × H15), 2.76 (m, 1H, H10), 3.32 (s, 3H, OMe), 3.34 (s, 3H, OMe), 3.31−3.34 (m, 1H, H11), 3.42−3.44 (m, 1H, H12), 4.23 (q, 2H, J = 7.0 Hz, OCH$_2$-*Me*), 4.32 (d, 1H, J = 9.0 Hz, H6), 5.16 (s, 1H, H7), 5.74 (d, 1H, J = 9.2 Hz, H9), 5.90 (t, 1H, J = 10.1 Hz, H5), 6.56 (t, 1H, J = 11.4 Hz, H4), 6.91 (d, 1H, J = 11.6 Hz, H3), 7.44 (s, 1H, H19), 8.73 (s, 1H, N22-H). MS *m*/*z* 640.5 (M^+ + Na). HRMS calculated for $C_{31}H_{43}N_3O_{10}Na$ (M^+ + Na): 640.2846. Found 640.2830.

General Procedure for the Synthesis of Geldanamycin Ureas Is Exemplified for 17-(Allylaminocarbonyl)amino-17-demethoxygeldanamycin 5. A solution of 17-amino-17-demethoxygeldanamycin (20 mg, 0.036 mmol) in EtOAc (10 mL) was treated with $Na_2S_2O_4$ (10%, 0.5 mL) at room temperature. After 2 h, the aqueous layer was extracted twice with EtOAc and the combined organic layers were dried over 18,21-dihydro-17-amino-17-demethoxygeldanamycin as a brown solid. This solid was dissolved in anhydrous THF (2 mL) under nitrogen atmosphere, and to the resulting solution was added allyl isocyanate (64 μL, 0.072 mmol) at room temperature. After 1 h, the solvent was removed under reduced pressure whereby the residue was dissolved in MeOH and the mixture was stirred overnight in the presence of silica. After filtration, the solvent was removed under reduced pressure and the residue was purified by flash chromatography to give 1-allyl-3-(17-geldanamycinyl)urea as a brick solid (5 mg, 22%). R_f = 0.38 in 80:15:5 CH_2Cl_2/EtOAc/MeOH. HPLC purity: 98.0%. ^{1}H NMR $CDCl_3$ δ 0.92 (d, 3H, J = 6.0 Hz, C10-Me or C14-Me), 0.95 (d, 3H, J = 6.9 Hz, C10-Me or C14-Me), 1.61−1.72 (m, 3H, 2 × H13, H14), 1.78 (s, 3H, C8-Me), 2.03 (s, 3H, C2-Me), 2.55 (m, 2H, 2 × H15), 2.74−2.78 (m, 1H, H10), 3.31 (s, 3H, OMe), 3.34 (s, 3H, OMe), 3.31−3.34 (m, 1H, H11), 3.43−3.45 (m, 1H, H12), 3.90 (br s, 2H, NHC*H*$_2$), 4.31 (d, 1H, J = 9.2 Hz, H6), 5.16 (s, 1H, H7), 5.20−5.29 (m, 3H, CHC*H*$_2$, NH), 5.79 (d, 1H, J = 9.4 Hz, H9), 5.84−5.94 (m, 2H, H5, C*H*CH$_2$), 6.57 (t, 1H, J = 11.8 Hz, H4), 6.92 (d, 1H, J = 11.4 Hz, H3), 7.28 (s, 1H, NHCO), 7.41 (s, 1H, H19), 8.79 (s, 1H, N22-H). MS *m*/*z* 731.7 (M^+ + Na). HRMS calculated for $C_{32}H_{44}N_4O_9$-Na (M^+ + Na): 651.3006. Found 651.2981.

General Procedure for the Synthesis of 17-Arylgeldanamycin Is Exemplified for 17-Phenyl-17-demethoxygeldanamycin 7a. A solution of 17-OTf-geldanamycin **6** (0.20 g, 0.30 mmol), cesium bromide (128 mg, 0.60 mmol), cesium fluoride (91 mg, 0.60 mmol), Pd(dba)$_2$ (43 mg, 0.075 mmol), and phenylboronic acid (73 mg, 0.60 mmol) in dioxane was heated at 40 °C for 12 h, whereupon it was cooled to room temperature and concentrated under reduced pressure to give the crude product. This solid was dissolved in EtOAc and washed with saturated $NaHCO_3$, dried over Na_2SO_4, and concentrated under reduced pressure. The crude material was purified by flash chromatography to give 17-phenylgeldanamycin as a yellow solid (90 mg, 50%). R_f = 0.49 in 80:15:5 CH_2Cl_2/EtOAc/MeOH. HPLC purity: 98.0%. ^{1}H NMR ($CDCl_3$) δ 0.71 (d, 3H, J = 6.5 Hz, C10-Me or C14-Me), 0.98 (d, 3H, J = 6.9 Hz, C10-Me or C14-Me), 1.53−1.63 (m, 3H, 2 × H13, H14), 1.79 (s, 3H, C8-Me), 2.04 (s, 3H, C2-Me), 2.35 (dd, 1H, J = 6.5, 13.2 Hz, H15), 2.54−2.60 (m, 1H, H15), 2.73−2.77 (m, 1H, H10), 3.31 (s, 3H, OMe), 3.34 (s, 3H, OMe), 3.35−3.37 (m, 1H, H11), 3.47−3.52 (m, 1H, H12), 4.36 (d, 1H, J = 8.9 Hz, H6), 5.24 (s, 1H, H7), 5.75 (d, 1H, J = 9.4 Hz, H9), 5.90 (t, 1H, J = 10.0 Hz, H5), 6.58 (t, 1H, J = 11.5 Hz, H4), 6.96 (d, 1H, J = 11.4 Hz, H3), 7.12−7.14 (m, 2H, Ph), 7.40−7.43 (m, 3H, Ph), 7.56 (s, 1H, H19), 8.68 (s, 1H, N22-H). MS *m*/*z* 629.6 (M^+ + Na). HRMS calculated for $C_{34}H_{42}N_2O_8Na$ (M^+ + Na): 629.2839. Found 629.2830.

17-(5-(Biotinamido)pentyl)amino-17-demethoxygeldanamycin 8. To 50 mg (0.152 mmol) of 5-(biotinamido)-pentylamine 4 in 3 mL of 15:1 THF/H_2O was added 28 mg (0.050 mmol) of geldanamycin **2** at room temperature. The reaction mixture was stirred overnight, quenched with water (50 mL), and extracted with 2 × 50 mL of EtOAc. The EtOAc extracts were combined, washed with 2 × 50 mL of H_2O and 1 × 50 mL of brine, dried ($MgSO_4$), and purified by silica gel flash chromatography to give 100 mg (0.114 mmol) of **8** in 75% yield. Mp = 143−147 °C. HPLC purity: 99.9%. ^{1}H NMR ($CDCl_3$) δ 0.99 (d, J = 7.0 Hz, 3H, C10-Me or C14-Me), 1.04 (d, J = 7.6 Hz, 3H, C10-Me or C14-Me), 1.46 (m, 4H, 2 × CH$_2$), 1.48 (m, 4H, 2 × CH$_2$), 1.72 (m, 6H, 3 × CH$_2$), 1.79 (m, 1H, CH), 1.80 (s, 3H, CH$_3$), 2.05 (s, 3H, CH$_3$), 2.26 (m, 4H, 2 × CH$_2$), 2.45 (d, 1H, CH$_2$), 2.65 (d, J = 13.1 Hz, 1H, CH$_2$), 2.77 (m, 2H, CH + CH$_2$), 2.95 (dd, J = 12.8, 5.0 Hz, 1H, CH$_2$), 3.20 (m, 2H, CH$_2$), 3.34 (s, 3H, OCH$_3$), 3.36 (s, 3H, OCH$_3$), 3.48 (t, J = 13.2 Hz, 2H, CH$_2$), 3.58 (m, 1H, CH), 4.30 (m, 2H, CH + NH), 4.50 (t, J = 7.6 Hz, 1H, CH), 4.92 (bs, 2H, 2CH), 5.21 (s, 1H, CH), 5.89 (m, 2H, 2CH═), 6.00 (bs, 2H, 2NH), 6.25 (bs, 1H, NH), 6.66 (t, J = 11.6 Hz, 1H, CH═), 7.00 (d, J = 11.6 Hz, 1H, CH═), 7.30 (s, 1H, CH═), 9.20 (s, 1H, CONH). MS *m*/*z* 880.2 (M^+ + Na). HRMS calculated for $C_{43}H_{64}N_6O_{10}SNa$ (M^+ + Na): 879.4302. Found 879.4296.

rHsp90 Competitive Binding Assay. An amount of 5 μg of purified rHsp90 protein (Stressgen, BC, Canada, No. SPP-770) in phosphated buffered saline (PBS) was coated on 96-well plates by incubating overnight at 4 °C. Unbound protein was removed, and the coated wells were washed twice with 200 μL of PBS. DMSO controls (considered as untreated samples) or test compounds were then added at 100, 30, 10, 3, 1, and 0.3 μM dilutions (in PBS), and samples in the plates were mixed for 30 s on a plate shaker and then incubated for 60 min at 37 °C. The wells were washed twice with 200 μL of

PBS, and 10 μM biotinylated geldanamycin (biotin-GM) was added and incubated for 60 min at 37 °C. The wells were washed again twice with 200 μL of PBS before the addition of 20 μg/mL streptavidin−phycoerythrin (Molecular Probes, Eugene, OR) and incubation for 60 min at 37 °C. The wells were washed again twice with 200 μL of PBS. Relative fluorescence units (RFU) was measured using a SpectraMax Gemini XS spectrofluorometer (Molecular Devices, Sunnyvale, CA) with an excitation of 485 nm and emission of 580 nm; data were acquired using SOFTmaxPRO software (Molecular Devices Corporation, Sunnyvale, CA). The background was defined as the RFU generated from wells that were not coated with Hsp90 but were treated with the biotin-GM and streptavidin-PE. Percent inhibition of binding for each sample was calculated from the background-subtracted values as follows:

$$\%\ \text{binding inhibition} = \frac{(\text{untreated RFU}) - (\text{sample RFU})}{\text{untreated RFU}} \times 100$$

IC_{50} was defined as the concentration of the compound at which there was 50% inhibition of the biotin-GM binding to rHsp90.

rHsp90 Conformational Assay. This was done by modification of a previously published method that utilized an environmentally sensitive fluorophore, 1,1′-bis(4-anilino-5-naphthalenesulfonic acid (bis-ANS) (Molecular Probes, Eugene, OR), to look at Grp94 conformational change (Wassenberg et al., 2000). rHsp90 (Stressgen, BC, Canada, No. SPP-770) at a final concentration of 2.5 μg in a total volume of 85 μL of buffer A (110 mM KOAc, 20 mM NaCl, 2 mM $Mg(OAc)_2$, 25 mM K-HEPES, pH 7.2, 100 μM $CaCl_2$) was added to 96-well plates. DMSO control or test compounds were added at 100, 30, 10, 3, 1, and 0.3 μM (10 μL of 10× stocks of each compound added), and the plates were mixed for 30 s on a plate shaker before incubation for 60 min at 37°C. Then to each well, 5 μL of 0.1 mM bis-ANS was added. The plate was covered with foil and mixed for 30 s on a plate shaker before incubation for 30 min at 37°C. Relative fluorescence units were measured using a SpectraMax Gemini XS spectrofluorometer (Molecular Devices Corporation, Sunnyvale, CA) at an excitation wavelength for bis-ANS at 393 nm, and emission wavelength was 484 nm. The data were acquired using the SOFTmaxPRO software (Molecular Devices Corporation, Sunnyvale, CA). The background was defined as the RFU generated from wells that did not get rHsp90 but to which the bis-ANS was added. The percent inhibition of conformational change, i.e., percent inhibition of bis-ANS activity for each sample, was calculated from the background-subtracted values as follows:

$$\%\ \text{inhibition of bis-ANS activity} = \frac{(\text{untreated RFU}) - (\text{sample RFU})}{\text{untreated RFU}} \times 100$$

IC_{50} was defined as the concentration of the compound at which there was 50% inhibition of bis-ANS activity.

Her-2 Degradation Assay. MCF7 breast carcinoma cells (ATCC) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 10 mM HEPES and plated in 24-well plates (50% confluent). Twenty-four hours later (cells are 65−70% confluent), test compounds were added at 1000 nM and six half-log dilutions down to 3 nM and incubated overnight for 16 h. The wells were washed with 1 mL of phosphate buffered saline (PBS), and 200 μL of trypsin was added to each well. After trypsinization was complete, 50 μL of FBS was added to each well. Then 200 μL of cells was transferred to 96-well plates The cells were pipetted up and down to obtain a single-cell suspension. The plates were centrifuged at 2500 rpm for 1 min using a Sorvall Legend RT tabletop centrifuge (Kendro Laboratory Products, Asheville, NC). The cells were then washed once in PBS containing 0.2% BSA and 0.2% sodium azide (BA buffer). PE conjugated anti-Her-2/neu antibody (Becton Dickinson, No. 340552) or PE conjugated anti keyhole limpet hemacyanin [KLH] (Becton Dickinson, No. 340761) control antibody was added at a dilution of 1:20 or 1:40, respectively (final concentration was 1 μg/mL), and the cells were pipeted up and down to form a single-cell suspension and incubated for 15 min. The cells were washed twice with 200 μL of BA buffer, resuspended in 200 μL of BA buffer, and transferred to FACSCAN tubes with an additional 250 μL of BA buffer. Samples were analyzed using an FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) equipped with an argon-ion laser that emits 15 mW of 488 nm light for excitation of the phycoerythrin fluorochrome; 10 000 events were collected per sample. A fluorescence histogram was generated, and the mean fluorescence intensity (MFI) of each sample was determined using Cellquest software. The background was defined as the MFI generated from cells incubated with control IgG-PE and was subtracted from each sample stained with the Her-2/neu antibody. Cells incubated with DMSO were always done as untreated controls because the compounds were resuspended in DMSO. The percent degradation of Her-2 was calculated as follows:

$$\%\ \text{Her-2 degradation} = \frac{\text{MFI Her-2 sample}}{\text{MFI Her-2 untreated cells}} \times 100$$

EC_{50} was defined as the concentration of the compound at which there was 50% degradation of the Her-2/neu protein.

Cell Lysate Binding Assay. MCF7 cell lysates were prepared in lysis buffer (20 mM Hepes, pH 7.3, 1 mM EDTA, 5 mM $MgCl_2$, 100 mM KCl) in a Dounce homogenizer and then incubated with or without test compound for 30 min at 4 °C, followed by incubation with biotin-GM linked to BioMagTM streptavidin magnetic beads (Qiagen) for 1 h at 4 °C. Tubes were placed on a magnetic rack, and the unbound supernatant was removed. The magnetic beads were washed three times in lysis buffer and boiled for 5 min at 95 °C in an SDS−PAGE sample buffer. Samples were analyzed on SDS protein gels, and Western blots were done for rHsp90. Bands in the Western blots were quantitated using the Bio-rad Fluor-S MultiImager, and the percent inhibition of binding of rHsp90 to the biotin-GM was calculated. The IC_{50} reported is the concentration of test compound needed to cause half-maximal inhibition of binding.

MTS Assay. Measurement of Cytotoxicity of Geldanamycin Derivatives. Cells were seeded in 96-well plates at 2000 cells/well and allowed to adhere overnight in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The final culture volume was 100 μL. Viable cell number was determined by using the Celltiter 96 AQ_{ueous} nonradioactive cell proliferation assay (Promega, Madison WI). The MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*- tetrazolium/PMS (phenazine methosulfate) solution was mixed at a ratio of 20:1, and 20 μL was added per well to 100 μL of culture medium. MTS is a tetrazolium dye that is converted to a formazan product by dehydrogenase enzymes of metabolically active cells,[31] which is measured at 490 nm absorbance after 2−4 h using a multiwell plate spectrophotometer. Background was determined by measuring the Abs 490 nm of cell culture medium and MTS−PMS in the absence of cells. The background value was subtracted from all values. The percent viable cells was calculated as follows:

$$\%\ \text{viable cells} = \frac{\text{Abs 490 sample}}{\text{Abs 490 nm untreated cells}} \times 100$$

IC_{50} was defined as the concentration that gave rise to 50% viable cell number.

Intravenous and Oral Pharmacokinetic Study in Mice. Female Balb/C mice were administered **2i** (dispersion formulation) as a 1 min intravenous infusion ($n = 3$) or by oral gavage ($n = 3$) at doses of 20 mg/kg (iv) or 100 mg/kg (oral). Blood samples were collected at intervals, and the total **2i** concentration in serum was determined using a reversed-phase HPLC−UV method with a 50 ng/mL limit of quantitation. Pharmacokinetic parameters were estimated by fitting

the mean **2i** concentration versus time data to a two-compartment model (WinNonlin).

HPLC Conditions. An Agilent 1100 HPLC system equipped with a photodiode array detector was used. A gradient method was used with mobile phase A consisting of water with 1% acetic acid and 0.5% triethylamine and mobile phase B consisting of acetonitrile with 1% acetic acid and 0.5% triethylamine. The content of mobile phase B was increased from 5% to 100% over 10 min, and the column was reequilibrated to the starting condition over 5 min (total run time = 15 min).The flow rate was 1.0 mL/min, and a Zorbax 300SB-C18 column was used (3.5 μM; 4.6 mm × 150 mm). Quantitation was based on UV absorbance at 254 nM compared to a standard.

Xenograft Studies in Mice. The human tumor cell lines A549 (non-small-cell lung carcinoma), H69 (small-cell lung cancer), and U87 (glioblastoma multiforme) were obtained from the American Type Tissue Culture Collection (ATCC) and maintained in DMEM supplemented with nonessential amino acids, sodium pyruvate, and HEPES. Female BALB/c nu/nu mice were obtained from Harlan and maintained in microisolator boxes. Recipient mice (five per group) were injected with 5×10^6 tumor cells on each flank, and tumors were allowed to become established (starting volumes of 60−160 mm^3) before onset of treatment. Hsp90 inhibitors were formulated in PETA (PEG 400/ethanol/Tween 80 mixed 6:3:1 in 0.05 M acetate buffer, pH 5). Animals received either PETA alone or **2b** or **2i** in PETA intraperitoneally (ip) at the doses and schedules indicated in the figure legends. Tumors were measured in three dimensions with calipers, and tumor volume was estimated using the algorithm volume (=length × width × height × $\pi/6$).

Supporting Information Available: R_f, HPLC purity, ^{1}H NMR, and HRMS data are provided for the compounds described in the manuscript. This material is available free of charge via the Internet at http://pubs.acs.org.

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