Potent Cytotoxic C-11 Modified Geldanamycin Analogues

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17-Allylamino-17-demethoxygeldanamycin (17-AAG) inhibits the activity of Hsp90, an important target for treatment of cancers. In an effort to identify analogues of geldanamycin (GDM) with properties superior to those of 17-AAG, we synthesized C-11 modified derivatives of GDM including ethers, esters, carbazates, ketones, and oximes and measured their affinity for Hsp90 and their ability to inhibit growth of human cancer cells. In accordance with crystal structures reported for complexes of GDMs with Hsp90, bulky groups attached to C-11 interfered with Hsp90 binding while smaller groups such as 11-*O*-methyl allowed Hsp90 binding. In addition, these analogues also showed in vitro cytotoxicity against human cancer cell lines. Esterfication of the 11-OH of 17-AAG eliminated Hsp90 binding in vitro. The readily hydrolyzed esters acted as prodrugs during the measurement of cytotoxicity. Thus, during these experiments, the esters were hydrolyzed, releasing 17-AAG. Several 11-*O*-methyl-17-alkylaminogeldanamycin analogues were identified with improved potency relative to 17-AAG.

Introduction

Heat shock protein 90 (Hsp90) has attracted attention as a molecular target for cancer therapeutics.[1−6] Geldanamycin (GDM[a]) (Scheme 1) was the first small molecule inhibitor identified for Hsp90.[7−9] The potent antiproliferative activities of GDM derivatives correlate with their abilities to deplete client proteins for Hsp90.[8,10−19] GDMs bind to the N-terminal domain ATP binding site of Hsp90, inhibiting the chaperone activity of the protein. This inhibition leads to the disruption of the Hsp90−client protein complex.[20−23] The unchaperoned client proteins are subsequently ubiquitinated and degraded by the proteosome. Because many of the Hsp90 client proteins are important in signal transduction and transcription, GDM and its analogues have potential utility in cancer chemotherapy. Indeed, 17-allylamino-17-demethoxygeldanamycin (17-AAG) and 17-[2-(dimethylamino)ethyl]amino-17-demethoxygeldanamycin (17-DMAG) (Scheme 1) are currently in multiple human clinical trials for the treatment of a range of malignancies.[24−27]

While numerous 17-aminogeldanamycin derivatives have been synthesized,[11,12,28−33] modification at the C-11 position has been limited. The 11-oxime analogues and 11-methylamino analogues reported by Schnur and co-workers showed weak activities in depleting the *erb*B-2 oncogene product (HER-2).[11] In an effort to identify GDM analogues with pharmacological properties superior to those of 17-AAG and 17-DMAG, we synthesized over 250 analogues exploring modifications at various positions. In this report, we describe several series of C-11 modified GDM derivatives and their biological activities.

Synthesis of Compounds

The 11-hydroxyl group of GDM or 17-alkylamino-17-demethoxygeldanamycin (**1**) can be alkylated to form ethers (**2** or **3**), acylated to form esters (**4**) or carbazates (**5**), or oxidized to give the 11-ketones (**6**), which in turn can be converted to amines (**7**) or oximes (**8**) (Scheme 2).

Selective alkylation of the 11-hydroxyl group in GDM proved to be difficult. When GDM was alkylated in the presence of 1 equiv of a strong base, deprotonation of the lactam NH occurred, resulting in 22-N-alkylation.[11] Treatment of GDM with more than 2 equiv of strong base such as potassium *tert*-butoxide (KO^{t}Bu) resulted in the loss of the C-7 carbamate. Methylation of GDM using methyl iodide plus silver oxide resulted in a mixture of products with mono- or dimethylation at the lactam and the 7-carbamate groups,[34] but the 11-*O*-methyl derivative was not observed.

After investigating a variety of alkylating conditions and protection schemes, we found that the 11-hydroxyl group of unprotected GDM and 17-alkylamino-17-demethoxygeldanamycins that do not bear any nucleophile in the 17-side chain could be regioselectively alkylated using the Meerwein salt and 1,8-bis(dimethylamino)naphthalene. For example, GDM was treated with 3 equiv of trimethyloxonium tetrafluoroborate and 3.5 equiv of 1,8-bis(dimethylamino)naphthalene in dichloromethane at 40 °C for 3 h to give 11-*O*-methylgeldanamycin (**2**) in ∼40% purified yield. 11-*O*-Methylgeldanamycin reacts with alkylamines to give 11-*O*-methyl-17-alkylamino-17-demethoxygeldanamycins (**3**).[28]

Preparation of other 11-*O*-ethers required protection of the lactam and the carbamate groups. Because of its reactivity toward nucleophiles, the 17-OMe group often interferes with reactions and purification. For the investigation of the 11-*O*-alkyl analogues, 17-azetidinyl-17-demethoxygeldanamycin was used as the starting material, since it does not have a reactive group on C-17. The 7-carbamoyl group was removed following a literature procedure (KO^{t}Bu in DMSO),[35] and the resulting 7-hydroxy group was protected as the *tert*-butyldimethylsilyl (TBS) ether (Scheme 3). The lactam NH was protected using the (2-trimethylsilylethoxy)methyl (SEM) group. With the 7-O and 22-N protected **11**, allylation of the 11-hydroxyl was carried out in a mixed solvent (1:1:1 v/v/v DMSO/THF/Et_2O) at −10 °C using KO^{t}Bu as the base and allyl bromide as the alkylating agent. The 11-*O*-benzyl ether was obtained under similar conditions using benzyl bromide. Attempts to alkylate using

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[a] Abbreviations: GDM, geldanamycin; 17-AAG, 17-allylamino-17-demethoxygeldanamycin; 17-DMAG, 17-[2-(dimethylamino)ethyl]amino-17-demethoxygeldanamycin.

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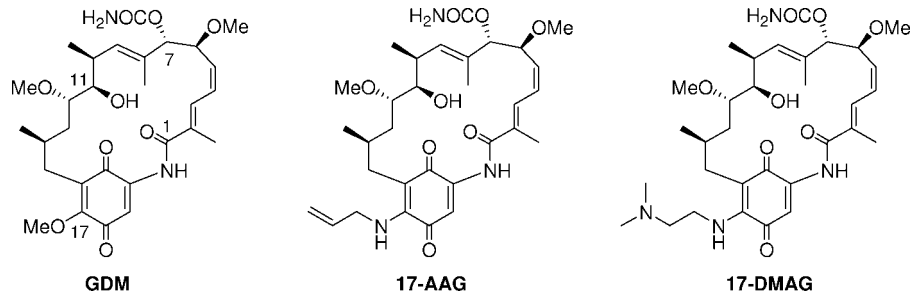
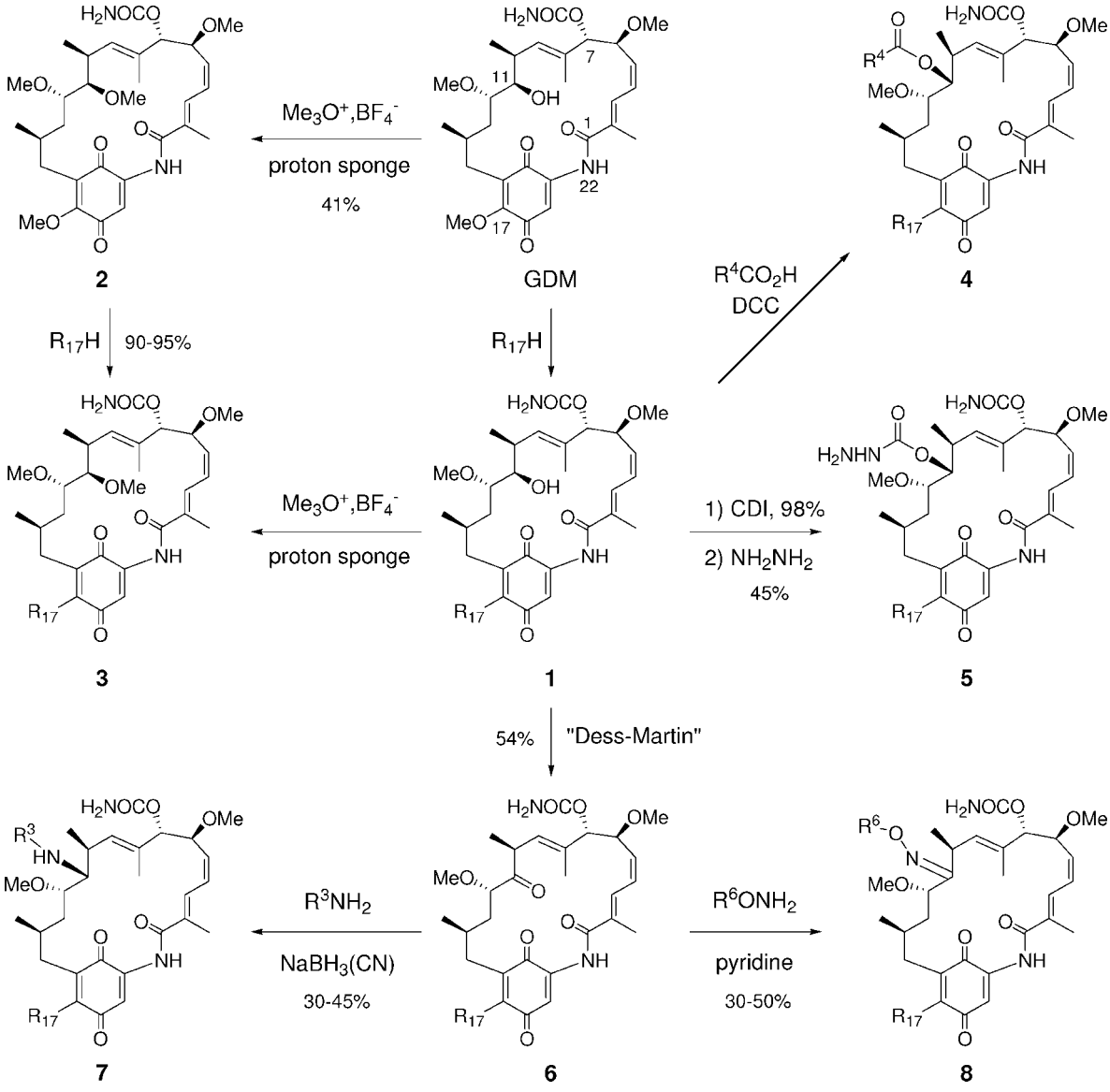
Scheme 1



Scheme 2

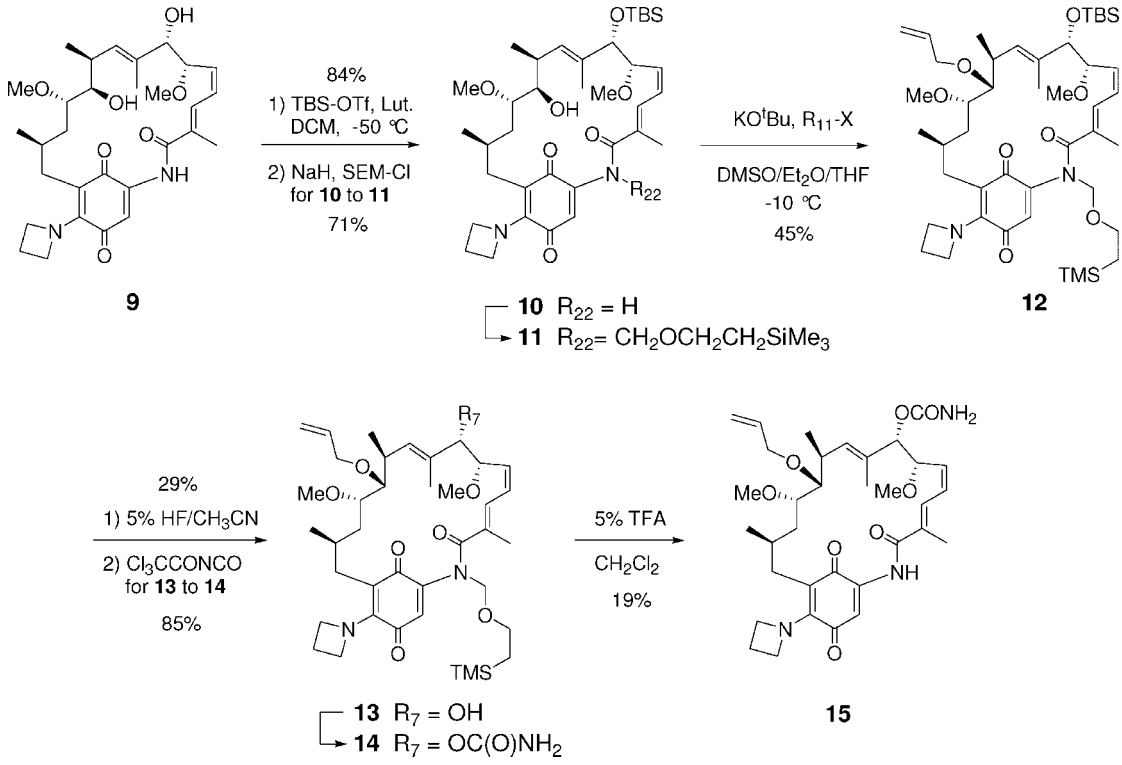


other bases or solvent systems resulted in either no reaction or decomposition. Alkylation using benzyl trichloroacetimidate also failed to give the desired product. Treatment of **12** with tetrabutylammonium fluoride or HF resulted in selective removal of the TBS group. The 7-carbamoyl group was then reinstalled using trichloroacetyl isocyanate.[36] Finally, the SEM group was removed using 5% TFA in dichloromethane to give **15**, accompanied by significant decomposition.

The 11-esters (**4**) were obtained by *N*,*N*′-dicyclohexylcarbodiimide (DCC) coupling of *tert*-butoxycarbonyl (Boc) protected amino acids with GDM or 17-alkylamino-17-demethoxygeldanamycins (**1**), followed by removal of the Boc group under acidic conditions.[37] Treatment of **1** with 1,1′-carbonyldiimidazole (CDI) gave the 11-(1-imidazolyl)formate derivatives, which reacted with hydrazine to give the 11-carbazate (**5**).

Oxidation of **1** using Dess−Martin periodinane gave the 11-ketone analogues (**6**).[11] Reductive amination of the 11-ketones resulted in the 11-amino derivatives (**7**). Schnur and co-workers reported (*R*)-configuration at C-11 for the 11-methylaminogeldanamycin derivatives.[11] By analogy, we assumed the C-11

Scheme 3



stereochemistry of the 11-amino derivatives we obtained was the same, since we used essentially the same reaction conditions. Reaction of the 11-ketones with hydroxylamine or alkyloxylamines yielded the 11-oxime or 11-oximinoethers (**8**). On the basis of the ^{13}C NMR chemical shifts of C-10 and C-12, the oximes were assigned as predominantly the (*E*)-isomer.[38]

Results and Discussion

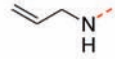
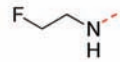
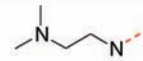
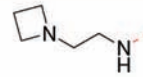
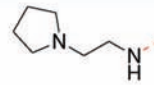
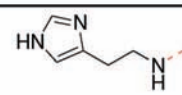
The analogues were tested for cytotoxicity in human tumor cell lines and for binding affinity for Hsp90. The IC_{50} for SKBr3 cell growth inhibition and the K_d for binding to recombinant human Hsp90 were measured using reported protocols.[28] The results for the 11-ether and 11-hydroxy analogues are shown in Table 1. The affinity of Hsp90 for the 11-ether analogues is typically 2- to 3-fold lower than that of the corresponding 11-hydroxy analogues. Exceptions to this general trend are the 11-*O*-methyl derivatives of GDM and 17-DMAG, which showed K_d values similar to those of GDM and 17-DMAG, respectively. The impact of the 11-*O*-methyl group on the cytotoxicity against SKBr3 cells varied depending on the side chain at C17. Compounds **2** and **3e** are among the most potent ($IC_{50} \approx 10$ nM) GDM analogues known to inhibit SKBr3 cell growth. In the 17-azetidinyl series, the 11-*O*-methyl and the 11-OH analogues (**3d** and **1d**) are both potent ($IC_{50} \approx 24$ nM), while the 11-*O*-allyl ether (**15**) is over 16-fold less potent. The 17-[2-(imidazol-4-yl)ethyl]amino analogues (**1h** and **3h**) have similar potency. The 11-*O*-methyl derivatives with other side chains at C17 (**3a**−**c**,**f**,**g**) are 2- to 8-fold less potent than the corresponding 11-OH analogues.

Snader and co-workers reported that 11-*O*-(4-aminobutanoyl)geldanamycin (**4g**) showed selective binding to Hsp90 compared to Grp94.[37] We therefore synthesized and studied the 11-ester analogues (**4a**−**f**) listed in Table 2. Although the glycinates (**4a**−**d**) showed weak activities in SKBr3 cell growth inhibition assays, they failed to bind to Hsp90 ($K_d > 25$ μM). We believe that the observed cytotoxicity of **4a**−**f** is due to hydrolysis of the 11-ester. While measurement of Hsp90 affinity can be accomplished in less than 1 h, the cytotoxicity assay uses a 72 h incubation of the test compounds with SKBr3 at pH 7.75, during which hydrolysis of the 11-esters was observed. In support of this conclusion, the cytotoxicity of 11-esters decreased as the half-life of the esters increased (Table 2). The 11-carbazate **5** is isosteric with the glycinate **4a**, but the carbazate is more resistant to hydrolysis. Consistent with this relative stability, **5** was inactive in our SKBr3 cell growth inhibition assay, although it showed a weak affinity for Hsp90.

11-Allylamino- and 11-benzylamino-17-methylamino-11-deoxy-17-demethoxygeldanamycin have been reported to have low activity.[11] We postulated that the positively charged amine at neutral pH may interact with Hsp90 unfavorably. Therefore, we investigated a series of 11-amino derivatives with amines having a range of basicity. As shown in Table 3, the amines have pK_a values[39−41] ranging from 5.7 to 9.2. The trifluoroethylamine derivative (**7d**) should exist as the free amine at neutral pH. However, all the derivatives we examined showed similarly weak binding affinity and cytotoxicity. We therefore conclude that the lack of activity of these compounds is not a result of an untoward electronic interaction and is more likely due to steric factors.

Four potent 17-alkylaminogeldanamycins (**1b**−**e**) were converted to the 11-ketone and 11-oxime analogues (**6a**−**d** and **8a**−**d** in Table 4). We found that all of these analogues were less active than the parent 11-hydroxy compounds in the Hsp90 binding and the cytotoxicity assays. With the fluoroethylamino side chain at C17, the oxime (**8c**) is much less active than the ketone (**6c**) in SKBr3 cell growth inhibition assays. With the allylamino, azetidinyl, and (dimethylaminoethyl)amino side chains at C17, the ketones and their oximes were equally cytotoxic. The affinities of the ketones for Hsp90 are similar to those of the corresponding oximes. The oximino ethers with neutral and positively or negatively charged side chains (**8e**−**i**) lost affinity for Hsp90. Although 17-DMAG-11-ketone (**6d**) and its oxime (**8d**) showed $IC_{50} \approx 100$

Table 1. Structures and Biological Activities for 11-Ether Analogues of GDM

Compound	OR^{11}	R^{17}-	IC_{50} (nM) [a]	K_d (μM) [b]
GDM	OH		41	0.67
2	OMe		9.1	0.49
1a [c]	OH		33	0.1
3a	OMe		84	0.32
1b [c] (17-AAG)	OH		33±10	1.3
3b	OMe		280	2.8
1c [c]	OH		17±3	0.8
3c	OMe		130	2.3
1d [c]	OH		24	1.4
3d	OMe		24±5	4.0
15	O-Allyl		390	2.9
1e [c] (17-DMAG)	OH		24±8	0.5
3e	OMe		11	0.8
1f [c]	OH		26	0.4
3f	OMe		130	1.0
1g [c]	OH		70	0.4
3g	OMe		160	1.0
1h [c]	OH		98	0.8
3h	OMe		60	2.0

[a] IC_{50} values were measured using the SKBr3 cells. The average value with an error bar is reported for results obtained in multiple experiments. A single value is given for results obtained from a single experiment. [b] K_d values were measured using full-length human Hsp90. [c] Previously reported compounds are included for reference.[28]

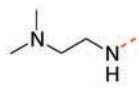
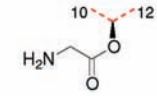
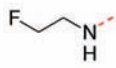
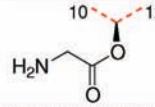
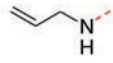
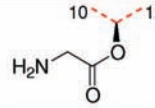
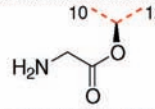
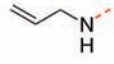
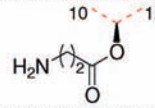
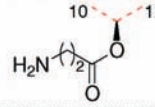
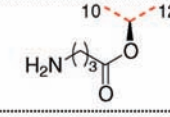
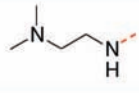
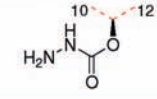
nM in the SKBr3 cell growth inhibition assay, the oximino ethers (**8e**−**i**) were markedly less active.

Both GDM-Hsp90[22] and 17-DMAG-Hsp90[23] cocrystal structures revealed a hydrogen bond between the 11-OH of GDM or 17-DMAG and Lys58 of Hsp90 where the 11-OH acts as an hydrogen bond acceptor. The structures also show a shallow binding pocket around C-11-OH. The Hsp90 binding affinities observed for the C-11-modified analogues are in accord with the structural information. Bulkier side chains at C-11 tended to decrease Hsp90 binding significantly. The 11-methoxy group is able to maintain the H-bond with Lys58; thus, the 11-*O*-methyl analogues showed only minimal disruption in binding to Hsp90. Although the 11-ketones and oximes are small in size, the change in geometry at C-11 from sp^3 to sp^2 hybridization may have caused slight changes in the conformation of the macrocycle that resulted in decreased Hsp90 affinity. This presumed conformational change together with *O*-alkyl group of the oxime (**8e**−**h**) resulted in total loss of Hsp90 binding. The carboxylate group in compound **8i** was designed to form a salt bridge with Lys58 of Hsp90, in the hope that this additional interaction would restore Hsp90 affinity. Indeed, **8i** gained binding (K_d > 25 uM) compared with **8f** and **8g**, while its binding is somewhat interfered by the bulky group at C-11. The loss of cytotoxicity of **8i** may be partially due to the inability of the carboxylate to cross the cell membrane.

As previously observed,[28] the K_d measured for in vitro binding of GDM analogues to purified Hsp90 is necessary but not sufficient for cytotoxicity, which is affected by factors such as cellular uptake[42,43] of the analogues and the state of the Hsp90 in a complex with other cofactors in cancer cells.[44] In cellular assays, the quinone species exist in redox equilibria with the hydroquinone species,[31] which might be another explanation for the discrepancy between binding and cytotoxicity.

In light of the potent cytotoxicity of the 11-*O*-methyl analogues against the human breast carcinoma SKBr3 cells, we tested these analogues (**3a**−**h**) against a panel of human cancer cell lines including MCF-7 (breast), SKOV3 (ovary), A549 (lung), CCRF-CEM (leukemia), as well as the multidrug-resistant (MDR) cell line CCRF-CEM/Taxol which is resistant to paclitaxel.[45] The results

Table 2. Structures, Biological Activities, and Hydrolysis Half Lives for the 11-*O*-Acyl Analogues of GDM

Compound	R^{17}-	C10-X-C12	IC_{50} (nM)	K_d (μM)	$T_{1/2}$ (h) [a]
4a			340	> 25	74
4b			110	> 25	nd [c]
4c			350	> 25	78
4d			480	> 25	95
4e			1800	> 25	130
4f			1700	> 25	200
4g			>1000	> 10	> 350 [b]
5			>1000	5	nd

[a] The hydrolysis kinetics were measured in 10 mM Tris buffer (pH 7.75). [b] No hydrolysis product observed. [c] Not determined.

Table 3. Structures and Biological Activities for 11-Amino Analogues[a]

Compd	R^3	pK_a of R^3NH_2[b]	IC_{50} (nM)	K_d (μM)
7a	H	9.2	415	>25
7b	FCH_2CH_2	9.0	390	>50
7c	F_2CHCH_2	7.5	370	2.9
7d	F_3CCH_2	5.7	270	4.0
7e	$F_3CCH_2CH_2$	8.9	830	>10
7f	$Me_2NCH_2CH_2$	6.0, 9.3	460	>50

[a] 11-Amino analogues derived from 17-DMAG (R_{17} = $Me_2NCH_2CH_2NH$). [b] References 39−41.

(Table 5) show that these analogues are active against MCF-7, SKOV3, A549, and CCRF-CEM tumor cell lines and less active against the MDR cell line CCRF-CEM/Taxol. However, compounds **3e**−**g** are 3- to 9-fold more potent than 17-AAG in inhibiting cell growth in all the tumor cell lines tested including the MDR cell line CCRF-CEM/Taxol except for compound **3g** in A549 cells, in which **3g** is equipotent to 17-AAG.

Conclusion

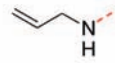
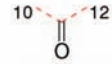
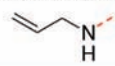
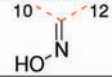
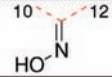
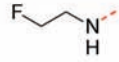
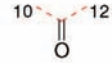
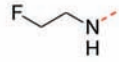
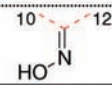
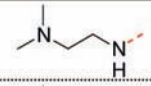
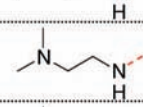
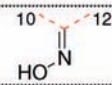
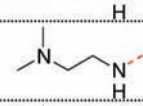
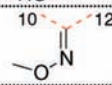
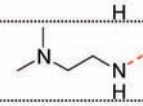
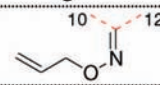
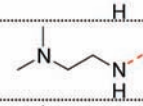
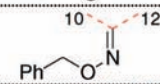
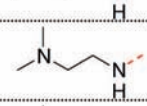
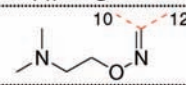
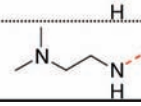
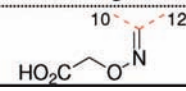
Our investigation of C-11 modified analogues of GDM and 17-alkylaminogeldanamycins showed that small groups at C-11 of GDMs are tolerated for Hsp90 binding activities. In accordance with crystal structures reported for the GDM-Hsp90 and 17-DMAG-Hsp90 complexes, bulky groups attached to C-11 interfered with Hsp90 binding. The 11-*O*-methyl analogues of a number of 17-alkylaminogeldanamycins showed a broad spectrum of in vitro cytotoxicity against human cancer cells. The activities observed for the C-11-modified analogues confirm a general finding that the in vitro binding of GDM analogues to purified Hsp90 is necessary but not sufficient for cytotoxicity.[28]

Esterfication of the 11-OH of 17-AAG eliminated Hsp90 binding, yet the more readily hydrolyzed esters were active in cytotoxicity assays during which they were converted to 17-AAG. If these compounds are found to have improved bioavailability or distribution, then it may be possible to use these compounds as prodrugs of 17-AAG. Finally, 11-*O*-methyl-17-alkylaminogeldanamycin analogues were identified with slightly improved cytotoxicity over 17-AAG against several cancer cell lines. Further investigation of the in vitro efficacy and pharmacological profiles of these compounds is needed to determine if these compounds hold any advantages over 17-AAG.

Experimental Section

Chemistry. Unless noted otherwise, all reagents and solvents were purchased from commercial sources and used without purification. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in $CDCl_3$ solution with a Bruker DRX 400

Table 4. Structures and Biological Activities of 11-Ketone and 11-Oxime Analogues of GDM

Compound	R^{17}-	C10-X-C12	IC_{50} (nM)	K_d (μM)
6a [a]			450	3.3
8a			350	> 10
6b [a]			260	6.4
8b [a]			310	> 25
6c			170	2.6
8c			>1000	> 25
6d			100	1.8
8d			95	>10 [b]
8e			550	>100
8f			400	>100
8g			480	>100
8h			2500	>100
8i			2700	> 25

[a] Known compounds synthesized as intermediates/references.[11] [b] K_d values were measured for the N-terminal domain of Hsp90.[46]

Table 5. IC_{50} (nM) for 11-*O*-Methyl-17-alkylaminogeldanamycin Analogues against a Panel of Cell Lines

Compd	MCF-7	SKOV3	A549	CCRF-CEM	CCRF-CEM/Taxol
3a	350	200	37	250	850
3b	760	1300	360	2500	3500
3c	58	460	67	960	2700
3d	63	110	29	420	2500
3e	50	43	23	58	390
3f	51	65	26	85	510
3g	76	69	62	160	520
3h	160	320	420	510	3000
17-AAG	230	220	68	540	2500

spectrometer. Chemical shifts were referenced to δ 7.26 and 77.0 ppm for ^1H and ^{13}C spectra, respectively. HRMS were obtained by FIA with manual peak matching on an Applied Biosystems Mariner TOF spectrometer with a turbo-ionspray source. HPLC purification was performed on a Varian Metasil Basic reversed-phase column, eluted with a gradient of acetonitrile in water (0.1% of acetic acid is added for compounds with charged side chains). Compound purity was determined by HPLC analysis on an Agilent 1100 system with a Zorbax Eclipse XDB-C18 column (2.1 mm × 150 mm, 3.5 μm). The analyte was eluted with a linear gradient of 20% acetonitrile (0.1% formic acid) to 95% acetonitrile (0.1% formic acid) at 0.3 mL/min over 10 min and detected by UV at 254 nm. All tested compounds had ≥95% purity.

11-*O*-Methylgeldanamycin (2). To a suspension of GDM (3.36 g, 6.0 mmol) in dichloromethane (300 mL) was added trimethyloxonium tetrafluoroborate (2.72 g, 18.4 mmol) and *N*,*N*,*N*′,*N*′-tetramethylnaphthalene-1,8-diamine (4.60 g, 21.5 mmol). After being stirred at 40 °C for 2 h, the reaction mixture was washed sequentially with 0.2 M HCl (150 mL), water (150 mL), saturated $NaHCO_3$ (150 mL), and brine (100 mL). The organic layer was concentrated and resuspended in diethyl ether (500 mL) and further washed with 0.2 M HCl (200 mL), water (200 mL), saturated $NaHCO_3$ (200 mL), and brine (100 mL). The solution was dried over $MgSO_4$, filtered, and evaporated to dryness. Purification by flash chromatography on silica gel (eluted using a gradient of ethyl acetate/dichloromethane containing 0.1% methanol) yielded 11-*O*-methylgeldanamycin (1.43 g, 41%). HPLC purity >98%. ^1H NMR ($CDCl_3$): δ 0.89 (d, J = 6.0 Hz, 3H), 1.03 (d, J = 6.4 Hz, 3H), 1.4−1.6 (m, 3H), 1.65 (s, 3H), 1.97 (s, 3H), 2.24 (dd, J = 8.8, 12.4 Hz, 1H), 2.58−2.70 (m, 2H), 3.29 (s, 3H), 3.30 (s, 3H), 3.32 (m, 1H), 3.44 (s, 3H), 3.51 (m, 1H), 4.06 (s, 3H), 4.42 (d, J = 7.2 Hz, 1H), 4.99 (br s, 2H), 5.49 (d, J = 9.2 Hz, 1H), 5.50 (s, 1H), 5.82 (dd, J = 7.2, 10.8 Hz, 1H), 6.48 (t, J = 11.6 Hz, 1H), 6.93 (m, 1H), 7.12 (s, 1H), 8.89 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 12.2, 13.9, 16.0, 21.5, 29.5, 32.5, 33.7, 36.5, 56.2, 57.4, 59.4, 61.5, 78.9,

79.4, 81.7, 83.3, 111.0, 125.7, 128.1, 130.5, 131.9, 134.5, 136.6, 137.8, 155.9, 156.9, 168.6, 183.9, 184.1. HRMS calculated for $C_{30}H_{42}N_2O_9Na$ ($M^+ + Na$): 597.2783. Found 597.2770.

General Procedure for the Synthesis of 11-*O*-Methyl-17-alkylamino-17-demethoxygeldanamycin (3a−h) As Exemplified by 11-*O*-Methyl-17-[2-(dimethylamino)ethyl]amino-17-demethoxygeldanamycin (3e). To a solution of **2** (1.28 g, 2.23 mmol) in 1,2-dichloroethane (20 mL) (DMSO was used when the amine is a salt, and triethylamine was used to release the amine) was added *N*,*N*-dimethylethylenediamine (0.5 mL, 4.6 mmol). After being stirred at 20 °C for 16 h, the mixture was diluted in ethyl acetate (50 mL), washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness. The crude product was purified by flash chromatography on silica gel, eluted using methanol/dichloromethane. The product was obtained as a purple solid (1.32 g, 94%). HPLC purity 99%. ^{1}H NMR ($CDCl_3$): δ 0.93 (d, J = 6.4 Hz, 3H), 1.07 (d, J = 6.8 Hz, 3H), 1.43 (m, 1H), 1.55−1.65 (m, 2H), 1.70 (s, 3H), 2.00 (s, 3H), 2.15 (dd, J = 9.2, 13.6 Hz, 1H), 2.26 (s, 6H), 2.55 (t, J = 6.0 Hz, 2H), 2.65 (m, 1H), 2.94 (d, J = 14.0 Hz, 1H), 3.32 (s, 3H), 3.35 (s, 3H), 3.36 (m, 1H), 3.45 (m, 1H), 3.52 (s, 3H), 3.55−3.65 (m, 2H), 4.48 (d, J = 7.6 Hz, 1H), 4.79 (br s, 2H), 5.49 (d, J = 9.2 Hz, 1H), 5.54 (s, 1H), 5.85 (dd, J = 7.6, 11.2 Hz, 1H), 6.51 (t, J = 10.6 Hz, 1H), 6.81 (br s, 1H), 7.01 (d, J = 10.8 Hz, 1H), 7.09 (s, 1H), 9.47 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 12.3, 14.1, 16.4, 21.6, 30.4, 33.6, 33.9, 36.4, 42.5, 44.8, 56.1, 57.4, 57.5, 59.8, 78.7, 79.5, 82.1, 83.5, 108.0, 108.7, 125.9, 127.8, 130.0, 132.1, 134.8, 136.4, 140.7, 145.1, 155.9, 168.7, 179.3, 184.4. HRMS calculated for $C_{33}H_{51}N_4O_8$ ($M^+ + H$): 631.3701. Found 631.3692.

11-*O*-Allyl-17-(1-azetidinyl)-17-demethoxygeldanamycin (15). To a solution of **9** (90 mg, 0.16 mmol) in dichloromethane (10 mL) at −60 °C under nitrogen atmosphere was added 2,6-lutidine (85 μL, 0.73 mmol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (83 μL, 0.36 mmol). The mixture was stirred at −60 °C for 30 min and then allowed to warm to room temperature over 1 h. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with saturated $NaHCO_3$ (20 mL) and saturated brine (20 mL). After the mixture was dried over $MgSO_4$, the crude product was purified by flash chromatography on silica gel, eluted using 1−10% methanol in dichloromethane to give **10** as a purple solid (95 mg). To a solution of **10** (600 mg, 0.9 mmol) in dry tetrahydrofuran (100 mL) was added sodium hydride in oil dispersion (400 mg). The mixture was stirred at room temperature until the color of the solution turned dark-blue (~2 h). Chloromethyl 2-trimethylsilylethyl ether (0.75 mL, 4.2 mmol) was added. After being stirred at room temperature for another hour, the mixture was diluted with ethyl acetate (150 mL) and carefully quenched with water (5 mL). The organic layer was washed sequentially with water (100 mL), saturated $NaHCO_3$ (100 mL), and brine (100 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. The crude product was purified by flash chromatography on silica gel, eluted using 15% ethyl acetate in dichloromethane. Compound **11** was obtained as a purple solid (510 mg).

To a solution of **11** (10 mg, 12 μmol) in 1:1:1 (v/v/v) DMSO/Et_2O/THF (0.9 mL) cooled at −10 °C was added allyl bromide (10 μL, 120 μmol). A 0.5 M solution of potassium *tert*-butoxide in 1:1 (v/v) DMSO/THF was added in 50 μL portions until the color of the solution started to turn brown from purple (~10 min). The reaction was quenched immediately by addition of ethyl acetate (10 mL) and 0.1 M HCl (1 mL). The organic layer was washed with saturated $NaHCO_3$ (10 mL) and brine (10 mL), dried over $MgSO_4$. The crude product was purified by flash chromatography on silica gel, eluted with 20% ethyl acetate in hexane to give **12** as a purple solid (5 mg).

To a solution of **12** (30 mg, 36 μmol) in acetonitrile (2 mL) cooled at 0 °C was added 5% HF in acetonitrile (0.5 mL). The mixture was stirred at 0 °C for 1 h and then allowed to warm to room temperature over 2 h. The reaction mixture was diluted with ethyl acetate (5 mL) and washed with saturated $NaHCO_3$ (5 mL) and brine (5 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness. The crude product was purified by flash chromatography on silica gel, giving **13** as a purple solid (~7 mg). This solid was dissolved in dichloromethane (1 mL) and stirred with trichloroacetyl isocyanate (20 μL) for 1 h at room temperature. The mixture was concentrated in vacuo and redissolved in methanol (1 mL). The solution was cooled to 0 °C, and potassium carbonate (20 mg) was added. After the mixture was stirred from 0 °C to room temperature over 2 h, dichloromethane (5 mL) was added. The mixture was washed with saturated $NaHCO_3$ (5 mL), and brine (5 mL) and dried over $MgSO_4$. The crude product was purified by flash chromatography on silica gel to give **14** as a purple solid (6.5 mg). HPLC purity 98%. ^{1}H NMR ($CDCl_3$) δ: 0.00 (s, 9H), 0.7−0.9 (m, 2H), 0.94 (d, J = 6.4 Hz, 3H), 1.02 (d, J = 6.4 Hz, 3H), 1.35 (s, 3H), 1.52−1.60 (m, 2H), 1.67 (s, 3H), 2.03 (s, 3H), 2.10 (m, 1H), 2.38 (m, 2H), 2.70 (AB, 1H), 3.07 (br s, 1H), 3.14 (m, 1H), 3.29 (s, 3H), 3.55−3.70 (m, 2H), 4.00 (m, 2H), 4.35−4.55 (m, 3H), 4.64 (m, 5H), 4.98 (d, J = 9.2 Hz, 1H), 5.09 (d, J = 10.4 Hz, 1H), 5.15−5.30 (m, 3H), 5.60 (d, J = 8.8 Hz, 1H), 5.90 (m, 1H), 6.32 (m, 2H), 6.45 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 0.5 (3C), 12.3, 14.1, 16.4, 17.9, 18.5, 21.5, 30.0, 33.6, 33.9, 36.3, 56.1, 57.6, 58.4 (2C), 68.2, 74.8, 75.2, 78.7, 79.6, 82.0, 83.5, 108.7, 109.4, 115.3, 126.1, 127.8, 129.9, 131.4, 132.1, 134.9, 136.3, 140.0, 145.7, 155.8, 169.0, 177.7, 186.3. HRMS calculated for $C_{40}H_{62}N_3O_9Si$ ($M^+ + H$): 756.4250. Found 756.4294.

To a solution of **14** (5 mg, 6.6 μmol) in dichloromethane (0.1 mL) cooled at 0 °C was added 5% (v/v) trifluoroacetic acid in dichloromethane (0.5 mL). After the mixture was stirred from 0 °C to room temperature over 30 min, it was diluted with ethyl acetate (2 mL) and quenched with $NaHCO_3$. The organic layer was washed with brine (2 mL) and dried over $MgSO_4$. The crude product was purified by HPLC in a C-18 column eluted with a gradient of acetonitrile in water, giving **15** as a purple solid (0.8 mg), 99% purity. HRMS calculated for $C_{34}H_{47}N_3O_8Na$ ($M^+ + H$): 648.3255. Found 648.3266.

Compounds 4a−g. Compounds **4c−f** were obtained from the National Cancer Institute. Compounds **4a**, **4b**, and **4g** were synthesized according to reported methods.[37]

11-*O*-(Hydrzinocarbonyl)-17-[2-(dimethylamino)ethylamino]-17-demethyoxygeldanamycin (5). To a solution of 17-DMAG (62 mg, 0.1 mmol) in 1,2-dichloroethane (5 mL) was added 1,1′-carbonyldiimidazole (81 mg, 0.5 mmol) and 4-dimethylaminopyridine (12 mg, 0.1 mmol). After the mixture was stirred at room temperature for 2 days, it was diluted in dichloromethane and washed with $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness. The crude product was purified by flash chromatography, giving the CDI-adduct as a purple solid (70 mg, 98%). To a solution of the CDI-adduct (15 mg, 0.02 mmol) in a mixture of 1,2-dichloroethane (0.5 mL) and ethanol (0.1 mL) was added hydrazine (3 μL, 0.1 mmol). After the mixture was stirred at room temperature overnight, it was evaporated to dryness. The crude solid was redissolved in water−acetonitrile and purified by HPLC on a C-18 column, eluted using a gradient of acetonitrile in water. The product was obtained of as a purple solid, 7 mg. HPLC purity 99%. ^{1}H NMR ($CDCl_3$) δ: 0.97 (d, J = 6.4 Hz, 3H), 0.99 (d, J = 6.4 Hz, 3H), 1.25 (m, 1H), 1.57 (m, 2H), 1.71 (s, 3H), 2.00 (s, 3H), 2.00 (m, 1H), 2.26 (s, 6H), 2.54 (t, J = 5.8 Hz, 2H), 2.81 (m, 2H), 3.32 (s, 6H), 3.40−3.80 (m, 5H), 4.50 (d, J = 6.8 Hz, 1H), 4.91 (br s, 2H), 5.34 (d, J = 10 Hz, 1H), 5.42 (s, 1H), 5.84 (dd, J = 7.6, 10.8 Hz, 1H), 5.91 (br s, 1H), 6.52 (t, 1H, J = 11.4 Hz), 6.80 (br s, 1H), 7.09 (s, 1H), 7.21 (br s, 1H), 9.41 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 12.3, 14.2, 15.9, 21.7, 30.4, 33.6, 33.9, 36.0, 42.5, 44.8, 56.1, 57.4, 57.5, 78.2, 79.0, 79.8, 80.2, 107.7, 108.4, 126.1, 128.1, 128.5, 133.1, 134.6, 135.8, 141.2, 145.2, 152.2, 155.6, 155.9, 169.1, 179.4, 184.3. HRMS calculated for $C_{33}H_{51}N_6O_9$ ($M^+ + H$): 675.7911. Found 675.7929.

General Procedure for the Synthesis of 11-Keto Compounds (6a−d) As Exemplified by 17-[2-(dimethylamino)ethylamino]-11-oxo-17-demethyoxygeldanamycin (6d). To a solution of 17-DMAG (250 mg, 0.4 mmol) in dichloromethane (15 mL) was added Dess−Martin periodinane (340 mg, 0.8 mmol). After the mixture was stirred at room temperature overnight, it was diluted in ethyl

acetate (20 mL) and washed sequentially with aqueous sodium thiosulfate, aqueous bicarbonate, and brine. The organic solution was dried over Na_2SO_4, filtered, and evaporated to dryness. The crude product was purified by HPLC on a C18 column, eluted using a gradient of acetonitrile in water containing 0.1% of acetic acid. The product was obtained as a purple solid (135 mg). HPLC purity 99%. ^{1}H NMR ($CDCl_3$) δ: 1.02 (d, J = 6.8 Hz, 3H), 1.26 (d, J = 7.2 Hz, 3H), 1.50 (m, 2H), 1.70−1.80 (m, 2H), 1.82 (s, 3H), 1.99 (s, 3H), 2.26 (s, 6H), 2.38 (m, 1H), 2.56 (m, 2H), 2.63 (m, 1H), 3.32 (s, 6H), 3.55 (m, 2H), 3.68 (m, 1H), 4.10 (m, 1H), 4.31 (d, J = 7.6 Hz, 1H), 4.93 (br s, 2H), 5.20 (s, 1H), 5.55 (d, J = 9.2 Hz, 1H), 5.82 (dd, J = 8.4, 10.8 Hz, 1H), 6.50 (t, J = 11.4 Hz, 1H), 6.84 (br s, 1H), 6.96 (d, J = 11.6 Hz, 1H), 7.14 (s, 1H), 9.27 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 12.3, 13.5, 16.6, 20.3, 31.2, 36.1, 41.9, 44.8(2 C), 57.7, 57.8, 80.6, 81.0, 83.2, 107.3, 108.3, 126.0, 127.1, 127.8, 134.8, 135.7, 136.1, 140.4, 146.0, 155.8, 168.4, 179.3, 184.2, 212.4. HRMS calculated for $C_{32}H_{47}N_4O_8$ (M^+ + H): 615.3388. Found 615.3358.

General Procedure for the Synthesis of 11-Oximes and Oximino Ethers (8a−i) As Exemplified by 17-[2-(Dimethylamino)ethylamino]-11-oxo-17-demethoxygeldanamycin-11-oxime (8d). To **6d** (30 mg, 0.049 mmol) in ethanol (4 mL) was added triethylamine (60 μL, 0.43 mmol) and hydroxylamine hydrochloride (30 mg, 0.4 mmol). After the mixture was stirred at room temperature for 3 h, TLC showed a complete reaction. The solvent was evaporated, and the residue was dissolved in chloroform and water. The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness. The crude product was purified by flash chromatography on silica gel to give product **8d** (10 mg) as a purple solid. HPLC purity 98%. ^{1}H NMR ($CDCl_3$) δ: 0.99 (d, J = 6.4 Hz, 3H), 1.18 (d, J = 7.2 Hz, 3H), 1.45 (m, 1H), 1.55 (m, 1H), 1.82 (s, 3H), 1.90 (m, 1H), 1.99 (s, 3H), 2.35 (s, 6H), 2.66 (m, 3H), 3.22 (s, 3H), 3.27 (s, 3H), 3.64 (m, 2H), 4.02 (m, 2H), 4.17 (d, J = 7.6 Hz, 1H), 4.93 (br s, 2H), 5.11 (s, 1H), 5.45 (d, J = 9.2 Hz, 1H), 5.75 (dd, J = 8.4, 10.8 Hz, 1H), 6.47 (t, J = 11.4 Hz, 1H), 6.92 (br s, 2H), 7.11 (s, 1H), 9.27 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 12.3, 13.1, 16.3, 19.2, 31.0, 31.2, 39.4, 41.8, 44.8(2 C), 57.3, 57.8, 58.0, 80.8, 82.0, 107.3, 108.3, 125.9, 127.3, 134.9, 135.9, 140.3, 140.6, 145.7, 156.2, 160.1, 168.6, 179.3, 184.4. HRMS calculated for $C_{32}H_{48}N_5O_8$ ($M + H^+$) 630.3497. Found 630.3493.

General Procedure for the Synthesis of 11-Amines (7a−f). To a solution of the amine HCl salt (0.63 mmol) in methanol (7 mL) was added methanolic solution of KOH (1 M) with stirring to free-base the amine. After the solution was stirred for 10 min under a nitrogen atmosphere, 11-keto-17-[2-(dimethylamino)ethylamino]-17-demethoxygeldanamycin (**6d**) (0.1 mmol) was added. The reddish solution was stirred at room temperature for 15 min. Sodium cyanoborohydride (0.47 mmol) was added, and the solution was heated at 50 °C for 2−3 days. Upon completion of the reaction, the solution was partitioned between chloroform and saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by HPLC on a C-18 column, eluted using a gradient of water/acetonitrile containing 0.1% (v) acetic acid to give purple solids.

Analytical Data for 11-Amines (7a−f) As Exemplified by 11-(2,2-Difluoroethyl)amino-17-[2-(dimethylamino)ethylamino]-11-deoxy-17-demethoxygeldanamycin (7c). ^{1}H NMR ($CDCl_3$) δ: 0.92 (d, J = 6.4 Hz, 3H), 1.01 (d, J = 6.8 Hz, 3H), 1.40 (m, 1H), 1.50 (m, 1H), 1.68 (m, 1H), 1.70 (s, 3H), 1.98 (s, 3H), 2.11 (m, 1H), 2.15 (s, 6H), 2.75 (m, 1H), 2.77 (m, 1H), 2.81 (t, J = 6.0 Hz, 2H), 2.97 (m, 2H), 3.01 (m, 1H), 3.24 (s, 3H), 3.30 (s, 3H), 3.50 (m, 1H), 3.64 (m, 1H), 3.75 (m, 1H), 4.36 (d, J = 6.8 Hz, 1H), 5.19 (br s, 2H), 5.36 (s, 1H), 5.60 (d, J = 8.4 Hz, 1H), 5.79 (m, 1H), 5.80 (tt, J = 4.4, 57 Hz, 1H), 6.49 (t, J = 11.2 Hz, 1H), 6.74 (m, 1H), 6.97 (m, 1H), 7.07 (s, 1H), 9.32 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 12.2, 13.4, 15.9, 20.6, 30.6, 30.8, 32.4, 36.0, 41.7, 44.3, 50.2 (t, $^2J_{C-F}$ = 23 Hz), 56.7, 57.2, 57.8, 59.9, 80. 2, 80.3, 80.5, 108.2, 108.9, 116.2 (t, $^1J_{C-F}$ = 241 Hz), 126.0, 127.9, 128.9, 133.2, 134.8, 136.3, 140.3, 145.4, 156.5, 168.8, 179.2, 184.6. HRMS calculated for $C_{34}H_{52}F_2N_5O_7$ ($M + H^+$) 680.3829. Found 680.3815.

Cell Growth Inhibition Assay. The human breast cancer cell lines SKBr3 and MCF-7, human lung adenocarcinoma A549, and human ovarian adenocarcinoma SKOV3 were obtained from the American Type Culture Collection (Manassas, VA). The CCRF-CEM human lymphoblastic leukemic cells and its paclitaxel-resistant subline (CCRF-CEM/Taxol) were kindly supplied by Dr. T. C. Chou (Memorial Sloan-Kettering Cancer Center). The SKBr3 and SKOV3 cells were maintained in McCoy's 5A modified medium (Invitrogen; Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) (Hyclone; Logan, UT) and 2 mM glutamine. Other cell lines were maintained in RPMI 1640 (Invitrogen) supplemented with 10% FBS (5% FBS for CCRF-CEM and its sublines) and 2 mM glutamine. The cells were incubated in humidified air with 5% CO_2 at 37 °C. Cells were seeded in duplicate in 96-well tissue culture microtiter plates at ~4000 cells per well and allowed to attach overnight. Serial 3-fold dilutions of test compounds were added, and the cells were incubated for 72 h. For SKBr3, MCF-7, SKOV3, and A549 cells, the cell viability was determined using the CellTiter-Glo luminescent cell viability assay (Promega; Madison, WI), and for other cells, the cell viability was determined using the CellTiter 96 AQ_{ueous} One solution cell proliferation assay (Promega). IC_{50} is defined as the concentration of drug required for inhibiting cell growth by 50%.

Hydrolysis of 11-Esters of GDM and 17-Allylaminogeldanamycin. Compounds (**4a**, **4c**−**4g**) were dissolved in DMSO to a concentration of 10 mM and diluted into 10 mM Tris, pH 7.75, to a final concentration of 50 μM. These solutions were incubated at room temperature (~22 °C) within the autosampler of an HP1090 HPLC system. The system automatically removed and analyzed samples for the disappearance of test compound and for formation of GDM or 17-allylaminogeldanamycin. HPLC analysis used an Inertsil ODS column (3 μm, 4.6 mm × 250 mm) and a mobile phase consisting of mixtures 5 mM NH_4OAc (buffer A) and 5 mM NH_4OAc in a 1:4 mixture of methanol/acetonitrile (buffer B). The column was equilibrated at 50 °C with 50% buffer B at 2 mL/min. Samples (10 μL) were injected onto the column, which was developed with a gradient to 100% buffer B over 10 min. Routine analysis monitored A_{304} and A_{332}. To verify the identity of hydrolysis products, mass spectrometric detection using a PE Sciex API100LC detector was used.

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