erbB-2 Oncogene Inhibition by Geldanamycin Derivatives: Synthesis, Mechanism of Action, and Structure–Activity Relationships

R. C. Schnur,* M. L. Corman, R. J. Gallaschun, B. A. Cooper, M. F. Dee, J. L. Doty, M. L. Muzzi, C. I. DiOrio, E. G. Barbacci, P. E. Miller, V. A. Pollack, D. M. Savage, D. E. Sloan, L. R. Pustilnik, J. D. Moyer, and M. P. Moyer

Pfizer Central Research, Groton, Connecticut 06340

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Overexpression of the *erbB-2* oncogene has been linked to poor prognosis in breast, ovarian, and gastric cancers. Naturally occurring benzoquinoid ansamycin antibiotics herbimycin A, geldanamycin (GDM), and dihydrogeldanamycin were found to potently deplete p185, the *erbB-2* oncoprotein, in human breast cancer SKBR-3 cells in culture. Chemistry efforts to modify selectively the ansa ring of GDM afforded derivatives with greater potency *in vitro* and *in vivo*. Analogs demonstrated inhibition of p185 phosphotyrosine in cell culture and *in vivo* after systemic drug administration to nu/nu nude mice bearing Fisher rat embryo cells transfected with human *erbB-2*. Functional group modification in the ansa ring was performed stereoselectively and regiospecifically without the need for protection strategies. Essential functional groups that were required for anti-*erbB-2* activity were the 7-carbamate and the 2,3-double bond. Modification of the functional groups at the other positions was permitted. Structure–activity relationships are described for 1–5-, 7–9-, 11-, 15-, and 22-substituted geldanamycins.

The $p185^{erbB-2}$ oncogene product has been observed in breast, stomach, and ovarian cancers and appears to be inversely correlated with survival.[1] Specific inhibitors that would block this function might be useful as anticancer agents. Geldanamycin analogs have recently been reported to cause depletion of the *erbB-2* gene product from human breast cancer cells, SKBR-3.[2] An accompanying article describes in greater detail the chemistry and antitumor activity for a series of geldanamycins modified on the quinone ring.[3] This article describes research aimed at discovering novel, potent, and selective inhibitors of $p185^{erbB-2}$ as useful antitumor agents through modifications of positions 2–15 and 22 of the ansa rings of geldanamycin (GDM) or 4,5-dihydrogeldanamycin (DHGDM).[4]

The molecular mechanism of action of ansamycin antioncogene agents has not been proven. Direct action of ansamycins on this growth factor receptor protein are still in question, since there are neither X-ray crystallographic studies on the *erbB-2* protein, either alone or in conjunction with an ansamycin inhibitor, nor data for inhibition by an ansamycin with *erbB-2* phosphorylation of an exogenous substrate. In the course of this research, analogs were prepared that could be used to probe the molecular target(s) of ansamycins via a photoaffinity labeling strategy. Detailed results of that research have been reported elsewhere.[5]

Chemistry

Ansa ring-modified geldanamycins were prepared according to Schemes 1 and 2. Reaction of GDM and DHGDM with amine nucleophiles afforded 17-amino-17-demethoxygeldanamycin derivatives **1a**–**h**.[3,6] These relatively stable materials were used for selective elaboration of the ansa ring functionalities employing a variety of reagents. While GDM and DHGDM undergo nearly all of these transformations under similar conditions, the 17-amino derivatives were chosen as starting materials because they were our ultimate targets for biological testing and they generally yielded products that were more easily isolated and purified.

Thus, treatment of **1a**,**b**,**g** with 1 equiv of potassium *tert*-butoxide in DMSO afforded the N-22 anion which, when treated with electrophiles, gave selectively 22-acyl or 22-alkyl species **2a**–**m**. In contrast, treatment of **1a**,**b** with 2.5 equiv of butoxide cleanly led to the 7-decarbamoyl analogs **4a**,**b**. Reaction of **1a**–**c** with weak bases and acylating agents selectively afforded 11-*O*-acyl products **3a**–**g**. If methoxide is employed in the reaction as the base, then ansa ring opening occurs to give the methyl ester **5a**.[7] The diols could be selectively oxidized with Dess–Martin reagent to give the 7-keto analogs, e.g., **4b** → **6a**. Excess oxidant gave the 7,11-diketone (not shown). Similarly, the diols reacted preferentially at the 7-hydroxy when combined with 1 equiv of acylating agent to give **7a**–**c**.

Geldanamycin has the 6(*S*)-, 7(*S*)-, 10(*S*)-, 11(*R*)-, 12(*S*)-, and 14(*R*)-stereochemical configuration as shown by the X-ray structure of **1a**.[8] Reaction of **1a**–**h** (11(*R*)-hydroxyl) with (diethylamino)sulfur trifluoride (DAST) at low temperature gave the 11(*S*)-fluoro derivatives **8a**–**f** as the major products. Their stereochemical assignments were made on the basis of NMR NOE determinations and coupling constants for **8f** and **1a** in conjunction with the X-ray structure for **1a** as illustrated in Table 2. The transposition of NOE effects seen for protons H-9 and H-12 when H-10 is irradiated suggested strongly that the fluorine at position 11 causes bond rotations in the conformation of the ansa ring from the C-9 to C-12 region. In a series of four ansamycins for which X-ray structures were obtained, the interatomic distance between O-11 and O-21 (quinone) averaged 3.29 Å. For **1a** this distance was 3.37 Å. The data support the hypothesis that a weak H-bond occurs between the two atoms. When O-11 is acylated there is a large downfield shift of the H-11

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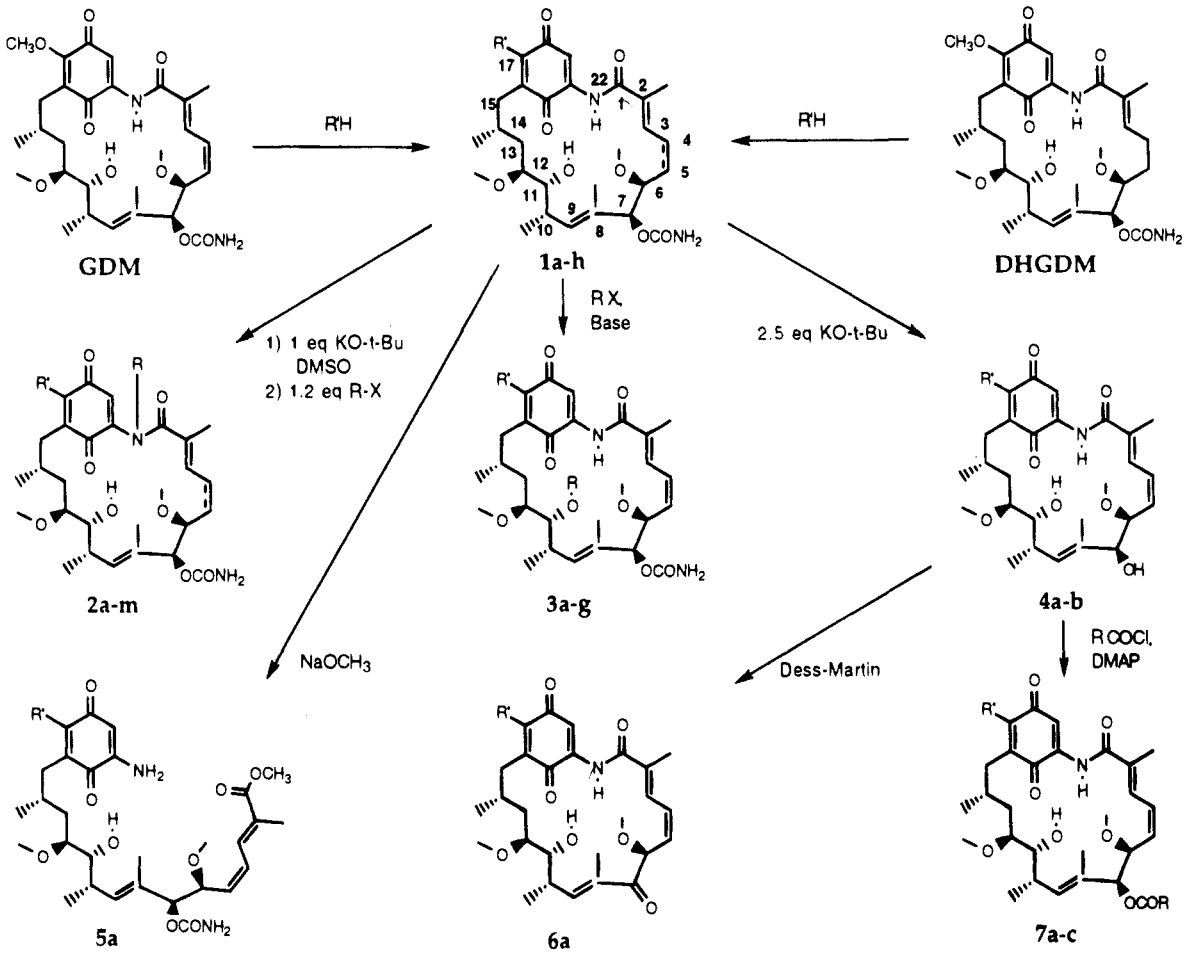
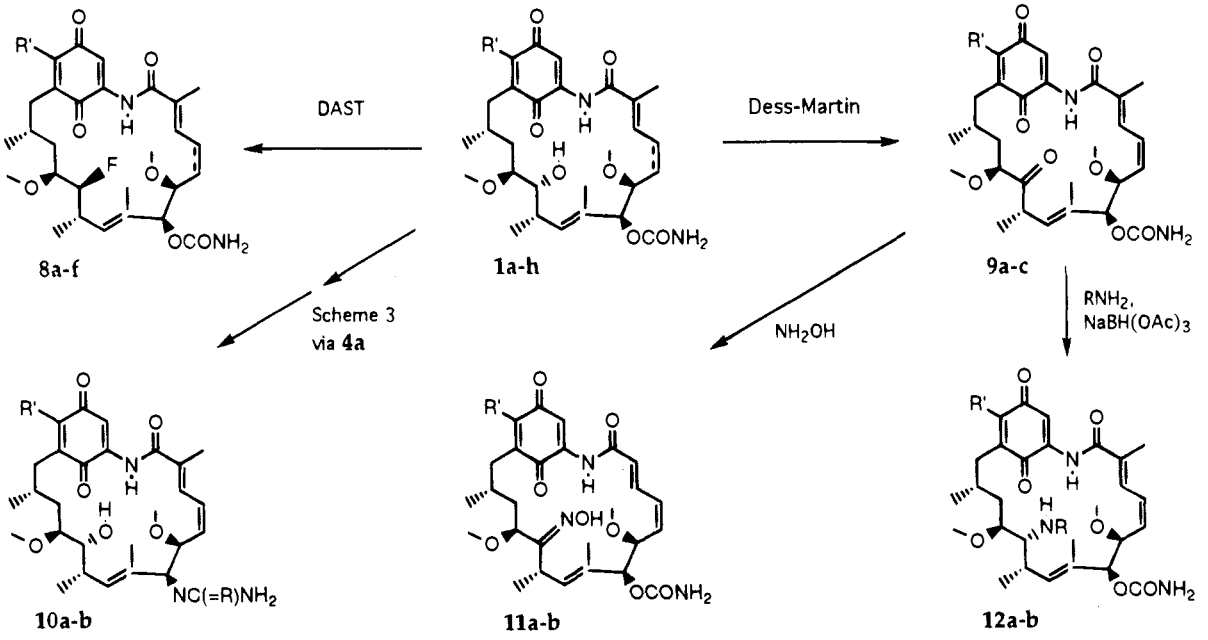
Scheme 1



Scheme 2



proton consistent with a conformational change concomitant with loss of the H-bond.

Treatment of **1a–e** with Dess–Martin reagent gave the 11-keto derivatives **9a–c**, precursors for the corresponding 11-oximes **11a,b** and 11-amines **12a,b**, via reductive amination. This reductive amination occurred stereoselectively and yielded presumably the 11(*R*)-amines.[9] Diol **4a** is an allylic alcohol and was converted stereoselectively to the 7(*S*)-ureas **10a,b** via a tandem sigmatropic rearrangement strategy as reported in detail by Schnur and Corman.[8]

Biology

Inhibition *in vitro* of p185$^{erbB\text{-}2}$ by geldanamycin analogs was determined using an assay which measured depletion of p185 protein upon treatment of cells with

Table 1. *In Vitro* and *in Vivo* Structure–Activity Relationships of Geldanamycin Analogs

compd[a]	R	R′	*in vitro*[b] IC_{50} (nM)	*in vivo*[c] % inhibtn	mp (°C)	yield (%)
HBM A			300	55[d]		
GDM		OCH_3	70	na		
DHGDM*		OCH_3	230			
1a		$-N(CH_2)_3-$	**23**	61	*f*	
1b		NH_2	37	59[d]	*f*	
1c		$NHCH_2CH{=}CH_2$	31	56	*f*	
1d		$NHCH(CH_3)_2$	60	87	*f*	
1e		$NHCH_3$	90	18	*f*	
1f*		$-N(CH_2)_3-$	14	44[d]	*f*	
1g*		NH_2	37	45	*f*	
1h*		$NHCH_2CH{=}CH_2$	51		*f*	
2a	phenacyl	NH_2	300		188–91	53
2b	3′,4′-dichlorophenacyl	NH_2	80	na	176–8	40
2c	3′-iodo-4′-azidophenacyl	NH_2	140		*h*	
2d	2′-methoxyphenacyl	NH_2	600	50	165–8	34
2e*	2′-methoxyphenacyl	NH_2	200		*g*	56
2f	4′-methoxyphenacyl	NH_2	1200		175–8	64
2g	4′-nitrophenacyl	NH_2	90		*g*	1
2h	1′-naphthacyl	NH_2	70	na	179–81	48
2i	2′-naphthacyl	NH_2	200	na	185–7	50
2j	4′-azidophenacyl	NH_2	230		165–7	15
2k	4′-azidophenacyl	$-N(CH_2)_3-$	1900		148–51	26
2l	2′-oxopropyl	NH_2	830		167–70	61
2m	2′-pyridylmethyl	NH_2	>3100		160–5	51
3a	$COCH_3$	$-N(CH_2)_3-$	70	78[e]	195 dec	56
3b	$CONHSO_2NHCH(CH_3)_2$	$-N(CH_2)_3-$	140	na	145–8	43
3c	$CONHSO_2N[(CH_2)_2]_2NCH_3$	$-N(CH_2)_3-$	190	na	147–9	42
3d	$CONH_2$	$-N(CH_2)_3-$	50	na	168–71	39
3e	$CONHSO_2N(CH_2)_3$	$NHCH_2CH{=}CH_2$	800		134–7	40
3f	$CONHSO_2NHCH(CH_3)_2$	$NHCH_2CH{=}CH_2$	1070		137–9	25
3g	$CONHSO_2N(CH_2)_2NCH_3$	$NHCH_2CH{=}CH_2$	>2500		133–5	26
4a		$-N(CH_2)_3-$	1290		*i*	
4b		NH_2	>3900		158–61	
5a		$-N(CH_2)_3-$	>3200		89–93	89
6a		$NHCH(CH_2)_3$	>3700		*g*	52
7a	$COCO_2H$	$-N(CH_2)_3-$	1000		152 dec	34
7b	$CONHCH_2CH_3$	$NHCH_2CH{=}CH_2$	>3300		112 dec	
7c	$COCH_2NH_2$	$NHCH_2CH{=}CH_2$	>3300		*g*	
8a		$-N(CH_2)_3-$	170	na	128 dec	40
8b*		$-N(CH_2)_3-$	40	na	104	51
8c		$NHCH_2CH{=}CH_2$	260	50	110–12	35
8d*		$NHCH_2CH{=}CH_2$	1200	na	84 dec	44
8e		NH_2	140	46[e]	>250	44
8f		$NHCH(CH_2)_2$	500		123–5	54
9a		$NHCH_2CH{=}CH_2$	220		112–8	96
9b		$-N(CH_2)_3-$	34	na	112–6	83
9c		$NHCH_3$	34	na	108–20	48
10a	S	$-N(CH_2)_3-$	1700		*h*	
10b	O	$-N(CH_2)_3-$	2600		*h*	
11a		$-N(CH_2)_3-$	1100	na	130–40	67
11b		$NHCH(CH_3)_2$	270		158–60	41
12a	$CH_2CH{=}CH_2$	$NHCH_3$	1000		123–5	71
12b	$CH_2C_6H_5$	$NHCH_3$	850		123–6	17

[a] Compounds marked with an asterisk (*) are 4,5-dihydrogeldanamycin derivatives. Typical experimental procedures for syntheses are given in detail for each of the structure types in the Experimental Section. C,H,N analyses are within the accepted 0.4% range except where indicated.[4] [b] As described in ref 2. [c] CD-1 (nu/nu) mice were implanted subcutaneously with FRE/*erbB-2* (Fisher rat embryo) cells and dosed at days 12–14 (~1 cm) ip with drug. At 3 h tumors were excised and extracted to isolate the p185 phosphotyrosine which was measured according to the procedure of ref 2. The recorded value is the percent inhibition obtained after dosing at 100 mg/kg unless otherwise indicated; na = not active. [d] At 400 mg/kg. [e] At 200 mg/kg. [f] Ref 3. [g] Glass. [h] Ref 5. [i] Ref 8.

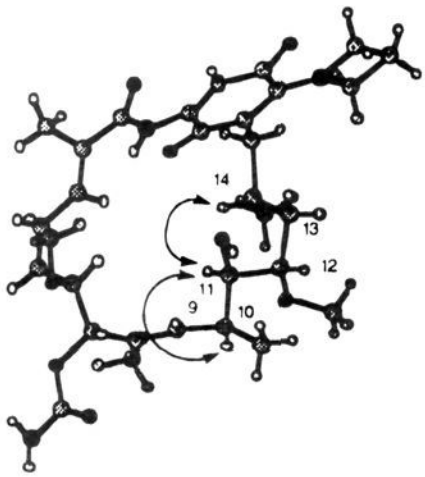
drugs as described by Miller.[2] Ansamycins were tested *in vivo* using a nude mouse assay bearing subcutaneous FRE/*erbB-2* tumors as described previously.[3]

Structure–Activity Relationships (SAR)

Modifications in the ansa ring were carried out in order to obtain compounds that were more potent than geldanamycin *in vitro* and *in vivo*[3] and useful for determining the mechanism by which geldanamycin caused p185 depletion. The compounds of our research embraced modifications in the ansa ring as well as at the 17-position. While a detailed description of that SAR can be found in the previous article,[3] we include here the results for a small set of derivatives at the 17-position because they were chosen for further modification due to their demonstrated superior *in vitro* potency. Their activity is included in Table 1 for comparison with other ansa ring derivatives. In general, the 17-azetidine and 17-allylamine derivatives were the more potent analogs among the variety of derivatives prepared, and thus, these examples are illustrated in this article.

A broad range of *in vitro* potency was observed when the N-H group at the 22-position was replaced by a substituent. Simple alkylation led to compounds >100-fold less potent, e.g., **2m** (or 22-*N*-methyl, not shown). However, if a keto function occupied the 2′-position of the alkyl group, as in phenacyl **2a** or in 2′-ketopropyl **2l**, then activity was restored, particularly with some

Table 2. NOE Connectivity of 11(*R*)-OH vs 11(*S*)-F Derivatives



1a
(X-ray)

proton irradiated	proton enhanced	% enhancement	
		1c, 11-OH(*R*)	**8f**, 11-F(*S*)
10	9	0.0	5.2
	11	3.6	8.6
	12	2.2	0.0
11	9	3.1	6.1
	10	4.9	4.2
	12	*a*	2.8
	14	5.8	0.0
12	11	*a*	2.8
	13	4.1	6.8

[a] NMR peaks too close together to observe NOE.

of the phenacyl analogs. This result is consistent with the interpretation that the 2′-keto group oxygen atom located on the 22-substituent can form an intramolecular H-bond preserving the overall ansa ring conformation found in the unsubstituted analogs, where the 22-NH points toward the inside of the ansa ring as shown in the X-ray.[8] Modeling studies are consistent with this conclusion. Without the intramolecular H-bonding forces, the bulk of even the small methyl group sterically interferes with the protons at the 3- and 6-positions and the 11-hydroxyl group, all of which lie on the inside of the ansa ring.

Some of the substituted phenacyl analogs, e.g., **2b**,**h**, were nearly as potent as GDM. 1′-Naphthacyl was slightly better than 2′-naphthacyl. Both DHGDM and GDM analogs **2d**,**e** were similarly active. In this series, in general, the best substituent at the 17-position was NH_2, **2j** vs **2k**, not azetidine, as seen in the 22-H series. Since there is an N-H proton on 17-amino analogs in contrast to the 17-azetidinyls, perhaps this is the result of tautomerism in the quinone to an *o*-iminoquinone yielding a free phenolic hydroxyl at position 21, which can H-bond to the 2′-ketone oxygen of the 22-side chain.

Acylation at the 22-position was facile. As an example, the 22-(4′-azidobenzoyl)-17-amino-17-demethoxygeldanamycin analog was prepared and found to be active. However, data suggest that the 22-acyl group was cleaved in cell culture. HPLC analysis was performed on the drug-treated cell culture after centrifugation to obtain a medium fraction and a cell pellet fraction. At 30 min in the medium fraction were found the acyl compound and the deacylated compound **1d**. In the pellet was found only **1d**. While this observation forms the basis for designing prodrugs in the GDM series, our principal aim was identification of an agent with desired biochemical properties. For this reason further exploration of N-22-acyl analogs was not advanced.

11-Hydroxy acylation and alkylation (data not shown) were performed with two concepts in mind. Either 11-position elaboration might result in uncovering an additional site for binding to the protein or the modification might block metabolism *in vitro* and/or *in vivo*, thus prolonging pharmacological half-life and perhaps increasing *in vivo* potency.[10] While a number of analogs were of similar potency *in vitro* to their unsubstituted precursors, e.g., **3e** vs **1c**, many were less potent, **3f**,**g**. In contrast, oxidation of the 11-OH to the ketone led to analogs with good potency, **9a**−**c**. These ketones were markedly more potent than their oximes **11a**,**b**. In addition, 11-amino-substituted analogs were prepared and found to be substantially less active than either the alcohols or ketones, exemplified by **12a**,**b**.

Unlike modifications at positions 3, 11, and 22, it appears that the 7-carbamate is required for potent activity. Even the minor modification of N-ethylation, **7b**, resulted in a 100-fold loss in activity. Cleavage of the carbamate led to loss of activity, **4a**,**b**. Oxidation of the diol selectively at the 7-position did not restore biological activity, **6a**. Only the oxalate half-ester **7a** and the ureas **10a**,**b** retained weak activity in the cellular assay.

The ansa ring of geldanamycin analogs can be cleanly opened with resultant loss of anti-p185 activity, **5a**. Similarly reduction of the 2,3-double bond, to afford the 2,3,4,5-tetrahydrogeldanamycin, afforded an inactive compound (IC_{50} > 3500 nM). 8,9-Epoxygeldanamycin has been previously prepared[11] and was shown to have an IC_{50} = 87 nM. During the synthesis of a few of the phenacylated analogs, particularly ones with electron-withdrawing groups, small amounts of 1-phenacylated analogs were detected and purified by flash chromatography (data not shown). These also were active in the *erbB-2* assay; however, their activity also may be the result of a prodrug phenomenon whereby these compounds, with their imidate functionality, are susceptible to hydrolytic reversion to **1a**−**h** when mixed in aqueous medium.

Attempts to selectively or doubly demethylate the 6-methoxy and the 12-methoxy were unsuccessful, thus preventing us from investigating the SAR at these positions. Herbimycins A and C had IC_{50}s of 300 and 270 nM, respectively, in our *in vitro* assay. These two analogs have a methoxy group at the 15-position, a hydrogen at the 17-position, and either a methoxy or a hydroxy at the 11-position, relatively. From this limited data, it appears that a minor modification at the 15-position did not greatly effect the bioactivity.

Ansa ring modifications were pursued as a strategy for obtaining $p185^{erbB-2}$ inhibitors with good *in vivo* activity, since it had been show that there was generally poor translation with simple 17-alkylamino derivatives from potent *in vitro* effects to potent *in vivo* responses. This approach appeared promising because there were numerous functional groups in the ansa ring that might be targets for metabolic alteration. *In vivo* testing was performed as described previously with FR*E*/*erbB-2* tumors in nude nu/nu mice.[3] Systemic administration

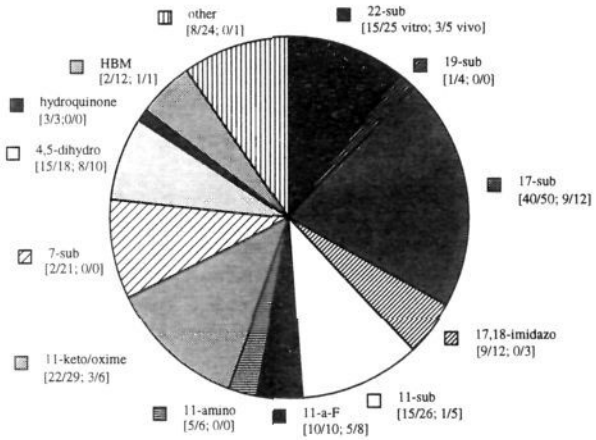


Figure 1. Ansamycin SAR *in vitro* and *in vivo*.

(ip) of GDM analogs reduced phosphorylated p185 in the tumors (see Table 1). GDM was inactive in this assay and was lethal at doses above 200 mg/kg. Generally analogs of structure **1a**−**g** were active, although only at very high doses for some. None of the other ansa ring modifications which retained their *in vitro* potency were more active than parents **1a**−**g**, though a few were active when tested at 100 mg/kg or higher, **2d**, **3a**, and **8c**,**e**. Many of the modification resulted in loss of *in vivo* activity, **2h**,**i**, **3b**−**d**, **8a**,**b**,**d**, and **9b**,**c**. Thus, there was no predictable SAR for *in vivo* activity, for example, only **2d** was active among four relatively potent N-22-phenacyl derivatives, and it was the least potent of the four tested. Only two of four fluoro analogs **8c**,**e** were active. Of four O-11-acyl analogs, only acetyl derivative **3a** was weakly active. This result may also involve hydrolase deacylation to generate the active azetidine **1a**. Neither did ketones **9b**,**c** demonstrate activity in spite of their high *in vitro* potency. Nevertheless, the sporadic appearance of *in vivo* activity among a diverse group of more potent ansa ring modifications supports the contention that metabolic alteration or conjugation of one of these functional groups is not likely to be the reason for poor *in vitro* to *in vivo* translation.

Thus, investigations into the SAR of the ansa ring of geldanamycins have resulted in variations of 11 of 16 centers, the 1−5-, 7−9-, 11-, 15-, and 22-positions. While many potent compounds were obtained from these modifications, none of the analogs resulted in *in vitro* or *in vivo* potency superior to the simple 17-substituted compounds **1a**−**h**. The pie chart, Figure 1, indicates the breadth and depth of our SAR investigations into 240 geldanamycin analogs, of which the highlights only are detailed above. Each pie section represents a category of ansamycin analogs where a specific position of the ansa ring has been modified and indicates a summary of the biological results. For example, of the 25 geldanamycin analogs that had an N-22-substituent, 15 were active in the SKBR-3 p-185^{erbB-2} assay, while three of five tested *in vivo* were found active [data in brackets].

The activity of **2b** suggested the possibility of incorporating a photoaffinity labeling moiety into the ansamycin. Thus, cold iodo azido analog **2c** was prepared. With an $IC_{50} = 140$ nM, this compound was sufficiently potent to expect labeling of protein in a cellular setting. Synthetic procedures have been reported[5] for the radioactive isomer. Mechanistic studies described binding of label to a single protein of 100 kDa after irradiation of drug-treated SKBR-3 cells.[5] These findings are significant because they do not support the interpretation of Fukazawa[12] that the ansamycin antibiotic herbimicin A exerts its tyrosine kinase inhibitory effect by directly binding irreversibly to reactive SH groups on the tyrosine kinases.

In conclusion, chemical modifications on the ansa ring of geldanamycins can be performed on a variety of centers with regioselectivity and stereospecificity without the necessity of protection of the other reactive functional groups. Modifications to the 1−5-, 7−9-, 11-, 15-, and 22-positions around the ansa ring have been investigated for their SARs. Geldanamycin and many of the modified analogs are potent inhibitors of the *erbB-2* oncogene function. A number of these derivatives also inhibit the oncogene product *in vivo* in contrast to geldanamycin which did not. The predictivity for translation from *in vitro* effects to *in vivo* activity was unreliable among potent inhibitors; however, *in vivo* activity was not demonstrated by the less potent members. The simple 17-substituted series was the subset of analogs that demonstrated the best *in vivo* activity. Alterations to the ansa ring functional groups, performed in an effort to find better *in vivo* activity, were largely unsuccessful. The SAR discovered in this research indicates that some structural features of the ansa ring are crucial for conferring specific *in vitro* biological properties (e.g., the 7-carbamate), while others provide the opportunity for structural modifications aimed at incorporating additional pharmacological (e.g., prodrug) or biochemical (e.g., photoaffinity labeling, **2c**) properties.

Further, our SAR studies indicate that the quinone moiety in the oxidized form is a requirement for inhibition.[2] It appears also that the SAR for oncogene inhibition intersects but is not parallel with that for their antibacterial activity and their RNA dependent DNA polymerase inhibition.[2] As shown by Miller et al., there is a strong dose−response correlation using analog **1c** between p185 protein depletion, the measurement used in these *in vitro* studies, with the inhibition of p185 autophosphorylation.[2] This extended to other members of the series when tested (data not shown) and was the basis for the use of p185 autophosphorylation inhibition as the end point in the *in vivo* assay.

Collateral studies have indicated that a photoactivatable radiolabeled geldanamycin analog, **2c**, that was potent for causing depletion of p185 in the SKBR-3 cells; did not bind covalently to the *erbB-2* protein upon nitrene formation induced by photoactivation but instead bound in cells to a single protein of approximate molecular weight 100 kDa as determined by electrophoretic mobility.[5] Active inhibitors from among the analogs reported above, when incubated in excess along with the photolabeling drug, were able to compete away the radiolabeling of p100 in a dose−response manner. In contrast, structurally similar inactive inhibitors lacked this ability completely. These results suggest that p100 mediates the depletion of p185^{erbB-2} by ansamycins, and therein lies the source of their regulation of signal transduction. Indeed p100 may play a wider role in mediation of signal transduction. Miller et al.[5] also showed that p100 was labeled in pp60^{src} transformed fibroblasts; therefore p100 may mediate the effect of ansamycins on p185^{erbB-2}, pp60^{src}, and other tyrosine kinases in a variety of cell types. Additional

studies continue on the nature of this interaction, the identification of the binding protein, and its role in the molecular mechanism by which geldanamycins inhibit signal transduction.

Experimental Section[13]

17-Azetidinyl-11-acetyl-17-demethoxygeldanamycin (3a). 17-Azetidinyl-17-demethoxygeldanamycin, **1a** (0.200 g, 0.341 mmol), was diissolved in 5 mL of methylene chloride in a flame-dried flask under nitrogen and treated with acetic anhydride (0.070 g, 0.683 mmol, 0.064 mL), (dimethylamino)-pyridine (DMAP) (Aldrich; 0.042 g, 0.341 mmol), and triethylamine (0.105 g, 1.04 mmol, 0.145 mL) at room temperature. After 3 h the mixture was diluted with 200 mL of methylene chloride, washed with 100 mL of water and 2 × 100 mL of brine, dried with sodium sulfate, filtered, and evaporated in vacuo to a residue, 0.30 g. This was flash chromatographed on 120 g of silica gel with 2.5% methanol:chloroform to afford pure product, 0.120 g, which was recrystallized from 10 mL of toluene, 0.080 g (37%): mp 195 °C dec; ^{1}H NMR ($CDCl_3$) δ 0.93 (m, 6H, 14-Me, 10-Me), 1.1–1.3 (m, 2H, H-13), 1.55 (m, 1H, H-14), 1.65 (s, 3H, 8-Me), 1.95 (s, 3H, 2-Me), 1.96 (s, 3H, acetyl CH_3), 2.0 (m, 1H, H-15), 2.35 (pent, J = 8 Hz, 2H, 3′-azetidine CH_2), 2.6–2.85 (m, 2H, H-15, H-10), 3.29 (s, 3H, OMe), 3.31 (s, 3H, OMe), 3.60 (sept, J = 8 Hz, 1H, isopropyl CH), 3.63 (m, 1H, H-12), 4.45 (d, J = 8 Hz, 1H, H-6), 4.55 (t, J = 8 Hz, 4H, 2′- and 4′-azetidine CH_2), 4.73 (br s, 2H, NH_2), 5.0 (m, 1H, H-11), 5.75 (d, J = 11 Hz, 1H, H-9), 5.41 (br s, 1H, H-7), 5.78 (t, J = 9 Hz, 1H, H-5), 6.46 (t, J = 9 Hz, 1H, H-4), 6.91 (s, 1H, H-19), 7.10 (m, J = 9 Hz, 1H, H-3), 9.34 (s, 1H, NH-22); MS m/z 650 (M^+ + Na); IR (KBr, cm^{-1}) 1735, 1685, 1640, 1590, 1540, 1480. Anal. ($C_{33}H_{45}N_3O_9$) C, H, N.

17-Amino-22-(2′-methoxyphenacyl)geldanamycin (2d). 17-Aminogeldanamycin (0.254 g, 0.465 mmol) was dissolved in 5 mL of anhydrous dimethyl sulfoxide in flame-dried glassware. Potassium *tert*-butoxide (0.054 g, 0.468 mmol) was added and the solution stirred at room temperature under nitrogen for 30 min. 2′-Methoxyphenacyl bromide (0.105 g, 0.458 mmol) was added and the solution stirred at room temperature for 2 h. This solution was diluted with ethyl acetate, washed with water and brine, dried over magnesium sulfate, filtered, and evaporated in vacuo. The resulting purple residue was flash chromatographed with silica gel eluted with 69% ethyl acetate:29% hexanes:1% methanol to give a purple solid, 0.110 g (35%): mp 165–168 °C; ^{1}H NMR ($CDCl_3$) δ 0.72 (d, J = 7 Hz, 3H, 14-Me), 0.84 (m, 1H, H-13), 1.03 (d, J = 7 Hz, 3H, 10-Me), 1.40 (s, 3H, 8-Me), 1.49 (m, 1H, H-13), 1.90 (dd, J = 12, 4 Hz, 1H, H-15), 2.03 (s, 3H, 2-Me), 2.24 (br m, 2H, H-10, H-14), 2.41 (s, 1H, 11-OH), 2.94 (m, 2H, H-12, H-15), 3.30 (s, 3H, OMe), 3.34 (s, 3H, OMe), 3.62 (d, J = 8 Hz, 1H, H-11), 3.97 (s, 3H, OMe), 4.28 (t, J = 10 Hz, 1H, H-6), 4.51 (d, J = 19 Hz, 1H, a-CH_2), 4.60 (br s, 2H, NH_2), 5.02 (d, J = 10 Hz, 1H, H-7), 5.10 (br s, 2H, NH_2), 5.20 (d, J = 9 Hz, 1H, H-9), 5.27 (t, J = 12 Hz, 1H, H-5), 5.86 (d, J = 17 Hz, 1H, a-CH_2), 5.98 (s, 1H, H-19), 6.40 (t, J = 13 Hz, 1H, H-4), 7.03 (m, 2H, aromatic), 7.14 (d, J = 12 Hz, 1H, H-3), 7.56 (m, 1H, aromatic), 7.90 (m, 1H, aromatic); MS m/z 694 (M^+); IR (KBr, cm^{-1}) 1732, 1678, 1661, 1584. Anal. ($C_{37}H_{47}N_3O_{10}\cdot H_2O$) C, H, N.

17-Azetidinyl-11-(aminocarbonyl)geldanamycin (3d). 17-Azetidinyl-17-demethoxygeldanamycin (0.200 g, 0.341 mmol) was dissolved in 5 mL of methylene chloride and cooled to 0 °C in a flame-dried flask under nitrogen. Sodium isocyanate (0.311 g, 0.4.78 mmol) and trifluoroacetic acid (0.545 g, 4.78 mmol, 0.368 mL) were added during 10 min. After stirring for 16 h at room temperature, the mixture was diluted with 200 mL of water and extracted with 3 × 150 mL of chloroform. The combined organic layers were washed with 2 × 100 mL of water, dried with sodium sulfate, filtered, and evaporated in vacuo to a residue, 0.236 g, which was flash chromatographed on 80 g of silica gel eluted with 30:69:1 hexane:ethyl acetate:methanol. Fractions containing pure product were evaporated, taken up in 2 mL of chloroform, and then precipitated with hexanes, 0.083 g (39%): mp 168–171 °C; ^{1}H NMR ($CDCl_3$) δ 0.98 (d, J = 8 Hz, 3H, 10-Me), 1.01 (d, J = 8 Hz, 3H, 14-Me), 1.3 (br m, 2H, H-13), 1.73 (s, 3H, 8-Me), 1.75 (br m, 1H, H-14), 2.02 (s, 3H, 2-Me), 2.15 (m, 1H, H-15), 2.40 (pent, J = 8 Hz, 2H, 3′-azetidine CH_2), 2.67 (m, 1H, H-15), 2.87 (m, 1H, H-10), 3.36 (s, 6H, OMe), 3.60 (m, 1H, H-12), 4.39 (br m, 2H, NH_2), 4.5 (br d, J = 9 Hz, 3H, H-6, NH_2), 4.64 (br t, J = 8 Hz, 6H, 2′- and 4′-azetidine CH_2, NH_2), 4.86 (m, 1H, H-11),5.35 (d, J = 12 Hz, 1H, H-9), 5.40 (br s, 1H, H-7), 5.83 (br t, J = 9 Hz, 1H, H-5), 6.54 (t, J = 9 Hz, 1H, H-4), 6.97 (s, 1H, H-19), 7.21 (br m, 1H, H-3), 9.36 (s, 1H, NH-22); MS m/z 629 (M^+); IR (KBr, cm^{-1}) 1720, 1686, 1648 1533, 1475. Anal. ($C_{32}H_{44}N_4O_9\cdot H_2O$) C, H, N.

17-(Allylamino)-11-[(isopropylsulfamyl)carbonyl]-geldanamycin (3f). 17-(Allylamino)-17-demthoxygeldanamycin (0.200 g, 0.341 mmol) was dissolved in 5 mL of methylene chloride and cooled to 0 °C in a flame-dried flask under nitrogen. Chlorosulfonyl isocyanide (0.080 mg, 0.564 mmol, 0.049 mL) was added dropwise during 10 min. After stirring for 1 h in the cold, isopropylamine (0.066 g, 1.13 mmol, 0.096 mL) was added and the reaction mixture allowed to warm to room temperature during 1 h. The reaction mixture was diluted with 100 mL of chloroform and extracted with 10 mL of water. The aqueous layer was back-extracted with 3 × 100 mL of chloroform. The pooled organic layers were extracted with 3 × 75 mL of 1 N NaOH. The combined basic layers were washed with 3 × 100 mL of chloroform and then acidified to pH 3 with 1 N HCl and extracted with 3 × 100 mL of chloroform. These latter organic extracts were pooled, washed with 2 × 100 mL of brine, dried with sodium sulfate, filtered, and evaporated in vacuo to a solid, 0.213 g. Flash column chromatography on silica gel eluted with 5:95 methanol:chloroform yielded pure target compound which was dissolved in 1 mL of chloroform, precipitated with hexane, filtered, and dried in vacuo, 0.061 g (25%): mp 137–139 °C; ^{1}H NMR ($CDCl_3$) δ 0.94 (d, 3H, J = 8 Hz, 10-Me), 0.98 (d, 3H, J = 8 Hz, 14-Me), 1.1 (m, 6H, isopropyl CH_3), 1.3–1.55 (br m, 2H, H-13), 1.65 (s, 3H, 8-Me), 1.70 (br m, 1H, H-14), 1.95 (s, 3H, 2-Me), 2.13 (m, 1H, H-15), 2.27 (dd, J = 7, 16 Hz, 1H, H-15), 3.00 (m, 1H, H-10), 3.25 and 3.27 (br s, 6H, OMe), 3.5 (m, 1H, isopropyl CH), 3.57 (br m, 1H, H-12), 4.05 (br t, 2H, allylic CH_2), 4.43 (br m, 1H, H-6), 4.7 (br m, 2H, NH_2), 4.9 (br s, 1H, NH) 5.02 (br d, J = 11 Hz, 1H, H-11), 5.2 (br d, 2H, vinylic CH_2), 5.38 (br m, 2H, H-7, H-9), 5.75 (t, J = 9 Hz, 1H, H-5), 5.85 (m, 1H, vinylic CH), 6.27 (br t, 1H, NH), 6.45 (t, J = 9 Hz, 1H, H-4), 7.03 (br m, 1H, H-3), 7.10 (s, 1H, H-19), 9.30 (s, 1H, NH-22); MS m/z 772 (M^+ + Na); IR (KBr, cm^{-1}) 1737, 1690, 1645. Anal. ($C_{35}H_{51}N_5O_{11}S\cdot 0.5H_2O$) C, H, N.

17-Azetidinyl-11-[(isopropylsulfamyl)carbonyl]-geldanamycin (3b). 17-Azetidinyl-17-demethoxygeldanamycin (0.200 g, 0.341 mmol) was dissolved in 5 mL of methylene chloride and cooled to 0 °C in a flame-dried flask under nitrogen. Chlorosulfonyl isocyanide (0.053 mg, 0.376 mmol, 0.033 mL) was added dropwise during 10 min. After stirring for 2 h in the cold, isopropylamine (0.044 g, 0.75 mmol, 0.064 mL) was added and the reaction mixture allowed to warm to room temperature during 1 h. The reaction mixture was diluted with 100 mL of methylene chloride and extracted with 2 × 100 mL of 1 N NaOH. The combined basic layers were washed with 3 × 150 mL of methylene chloride and then acidified to pH 3 with 1 N HCl and extracted with 3 × 150 mL of methylene chloride. These latter organic extracts were pooled, dried with sodium sulfate, filtered, and evaporated in vacuo to a solid, 0.121 g, which was dissolved in 1 mL of methylene chloride, precipitated with hexane, filtered, and dried in vacuo, 0.110 g (43%): mp 145–148 °C; ^{1}H NMR ($CDCl_3$) δ 0.90 (d, J = 8 Hz, 3H, 14-Me), 0.96 (d, J = 8 Hz, 3H, 10-Me), 1.14 (d, J = 8 Hz, 6H, isopropyl CH_3), 1.3 (m, 1H, H-13), 1.5 (m, 1H, H-13), 1.6 (m, 1H, H-14), 1.64 (s, 3H, 8-Me), 1.94 (s, 3H, 2-Me), 2.0 (m, 1H, H-15), 2.36 (p, J = 8 Hz, 2H, 3′-azetidine CH_2), 2.73 (dd, J = 8, 16 Hz, 1H, H-15), 2.9 (m, 1H, H-10), 3.25 (s, 3H, OMe), 3.27 (s, 3H, OMe), 3.52 (sept, J = 8 Hz, 1H, isopropyl CH), 3.63 (m, 1H, H-12), 4.43 (m, 1H, H-6), 4.57 (t, J = 8 Hz, 4H, 2′- and 4′-azetidine CH_2), 4.78 (br s, 2H, NH_2), 5.0 (br s, 1H, H-11), 5.86 (m, 2H, H-7, H-9), 5.75 (t, J = 9 Hz, 1H, H-5), 6.45 (t, J = 9 Hz, 1H, H-4), 6.9 (s, 1H, H-19), 6.95 (m, J = 9 Hz, 1H, H-3), 7.45 (m, 1H, NH), 9.35 (s, 1H, NH-22); MS m/z 772 (M^+ + Na); IR (KBr, cm^{-1}) 1735, 1685, 1645. Anal. ($C_{35}H_{51}N_5O_{11}S\cdot 1.25H_2O$) C, N; H: calcd, 6.98; found, 6.54.

17-Azetidinyl-11-[[(4′-methyl-1′-piperazinyl)sulfamyl]carbonyl]geldanamycin (3c). 17-Azetidinyl-17-demethoxygeldanamycin (0.200 g, 0.341 mmol) was dissolved in 5 mL of methylene chloride and cooled to 0 °C in a flame-dried flask under nitrogen. Chlorosulfonyl isocyanide (0.053 mg, 0.376 mmol, 0.033 mL) was added dropwise during 10 min. After stirring for 1 h in the cold, *N*-methylpiperazine (0.075 g, 0.75 mmol, 0.083 mL) was added and the reaction mixture allowed to warm to room temperature during 1 h. The reaction mixture was diluted with 100 mL of chloroform, extracted with 100 mL of water and 2 × 100 mL of brine, dried with sodium sulfate, filtered, and evaporated in vacuo to a solid, 0.280 g. This was flash chromatographed on silica gel with 10% methanol:chloroform affording pure product, 0.114 g (42%): mp 147–149 °C; ^{1}H NMR ($CDCl_3$) δ 1.11 (d, J = 8 Hz, 3H, 14-Me), 1.21 (d, J = 8 Hz, 3H, 10-Me), 1.55 (m, 1H, H-13), 1.72 (m, 1H, H-13), 1.83 (m, 1H, H-14), 1.86 (s, 3H, 8-Me), 2.2 (br s, 4H, 2-Me, H-15), 2.53 (s, 3H, N-CH_3), 2.60 (br t, J = 8 Hz, 2H, 3′-azetidine CH_2), 2.70 (br s, 4H, piperazine CH_2), 2.9–3.1 (m, 2H, H-10, H-15), 3.53 (s, 6H, OMe), 3.86 (m, 1H, H-12), 4.69 (br s, 1H, H-6), 4.82 (t, J = 8 Hz, 4H, 2′- and 4′-azetidine CH_2), 5.15 (br s, 2H, NH_2), 5.72 (br s, 1H, H-7), 5.57 (br d, 1H, H-11), 5.66 (br s, 1H, H-9), 6.00 (t, J = 9 Hz, 1H, H-5), 6.68 (t, J = 9 Hz, 1H, H-4), 7.15 (s, 1H, H-19), 7.24 (br s, 1H, H-3), 7.45 (s, 1H, NH), 9.60 (s, 1H, NH-22); MS *m/z* 813 (M^+ + Na); IR (KBr, cm^{-1}) 1738, 1688, 1646, 1583, 1471. Anal. ($C_{37}H_{54}N_6O_{11}S$·H_2O) C, H, N.

17-(Allylamino)-11-[(azetidinylsulfamyl)carbonyl]geldanamycin (3e). 17-(Allylamino)-17-demethoxygeldanamycin (0.200 g, 0.341 mmol) was dissolved in 5 mL of methylene chloride and cooled to 0 °C in a flame-dried flask under nitrogen. Chlorosulfonyl isocyanide (0.053 mg, 0.376 mmol, 0.033 mL) was added dropwise during 10 min. After stirring for 1 h in the cold, azetidine (0.043 g, 0.75 mmol, 0.051 mL) was added and the reaction mixture allowed to warm to room temperature during 1 h. The reaction mixture was evaporated to a residue and flash column chromatographed on 60 g of silica gel eluted with 30:69:1 hexane:ethyl acetate:methanol to yield pure target compound which was dissolved in 1 mL of chloroform, precipitated with hexane, filtered, and dried in vacuo, 0.102 g (40%): mp 134–137 °C; ^{1}H NMR ($CDCl_3$) δ 0.92 (d, 3H, J = 8 Hz, 10-Me), 1.01 (d, 3H, J = 8 Hz, 14-Me), 1.4 (br m, 1H, H-13), 1.5 (br m, 2H, H-13, H-14), 1.65 (s, 3H, 8-Me), 1.95 (s, 3H, 2-Me), 2.13 (m, 1H, H-15), 2.05–2.2 (m, 3H, H-15, azetidine 3′-CH_2), 2.78 (dd, J = 6, 15 Hz, 1H, H-15), 2.93 (m, 1H, H-10), 3.26 (s, 3H, OMe), 3.28 (s, 3H, OMe), 3.63 (br m, 1H, H-12), 3.95–4.05 (br m, 6H, allylic CH_2, azetidine 2′- and 4′-CH_2), 4.45 (br s, 1H, H-6), 4.7 (br m, 2H, NH_2), 5.02 (br d, J = 11 Hz, 1H, H-11), 5.2 (m, 2H, vinylic CH_2), 5.4 (br m, 2H, H-7, H-9), 5.73–5.93 (m, 2H, H-5, vinylic CH), 6.25 (br t, 1H, NH), 6.45 (t, J = 9 Hz, 1H, H-4), 7.03 (br m, 1H, H-3), 7.10 (s, 1H, H-19), 9.32 (s, 1H, NH-22); MS *m/z* 769 (M^+ + Na); IR (KBr, cm^{-1}) 1734, 1691, 1645 1579, 1474. Anal. ($C_{35}H_{49}N_5O_{11}S$·$0.75H_2O$) C, H, N.

17-Azetidinyl-11-[(piperazinylsulfamyl)carbonyl]-17-demethoxygeldanamycin (3g). 17-Azetidinyl-17-demethoxygeldanamycin (0.50 g, 0.854 mmol) was dissolved in 5 mL of methylene chloride and cooled to 0 °C in a flame-dried flask under nitrogen. Chlorosulfonyl isocyanide (0.133 mg, 0.939 mmol, 0.082 mL) was added dropwise during 10 min. After stirring for 1 h in the cold, piperazine (0.162 g, 1.88 mmol) was added and the reaction mixture allowed to warm to room temperature during 1 h. The reaction mixture was evaporated to dryness and flash chromatographed on 200 g of silica gel with 20% methanol chloroform affording pure product which was dissolved in 5 mL of chloroform and precipitated with 150 mL of hexane, 0.161 g (24%): mp 180–182 °C; ^{1}H NMR ($CDCl_3$) δ 0.86 (m, 3H, 14-Me), 0.9 (m, 3H, 10-Me), 1.6 (s, 3H, 8-Me), 1.94 (br s, 2-Me), 2.35 (pent, J = 8 Hz, 2H, 3′-azetidine CH_2), 3.25 (br s, 6H, OMe), 3.64 (m, 1H, H-12), 4.46 (br s, 1H, H-6), 4.6 (t, J = 8 Hz, 4H, 2′- and 4′-azetidine CH_2), 6.43 (br t, 1H, H-4), 6.9 (s, 1H, H-19), 9.35 (s, 1H, NH-22), other protons observed but not well defined nor assignable; MS *m/z* 799 (M^+ + Na); IR (KBr, cm^{-1}) 1734, 1689, 1646, 1600, 1471. Anal. ($C_{33}H_{52}N_6O_{11}S$·$2H_2O$) C, H, N.

17-Amino-7-decarbamoyl-17-demethoxygeldanamycin (4b). This compound was prepared from **1c** by treatment with 2 equiv of potassium *tert*-butoxide in dimethyl sulfoxide at room temperature according to the method of Schnur and Corman.[8] Anal. ($C_{27}H_{38}N_2O_7$·$0.5H_2O$) C, H, N.

Methyl 17-Azetidinyl-17-demethoxygeldanamycinate (5a). **1a** (1.00 g, 1.707 mmol) was dissolved in 50 mL of methanol and treated with sodium methoxide (0.92 g, 1.707 mmol) at room temperature for 16 h. The solvent was removed in vacuo and the residue dissolved in 300 mL of ethyl acetate, washed with 2 × 100 mL of water and 3 × 100 mL of brine, dried with magnesium sulfate, filtered, and evaporated in vacuo to a purple residue. This was recrystallized from ethyl acetate: hexanes, 0.933 g (89%): mp 89–93 °C; ^{1}H NMR ($CDCl_3$) δ 0.62 (d, J = 8 Hz, 3H, 14-Me), 0.85–0.95 (br m, 1H, H-13), 0.90 (d, J = 8 Hz, 3H, 10-Me), 1.35–1.5 (m, 1H, H-13), 1.52 (s, 3H, 8-Me), 1.73 (s, 3H, 2-Me), 2.1–2.45 (m, 5H, H-10, H-15, 3′-azetidine CH_2), 3.05 (m, 1H, H-12), 3.17 (s, 3H, OMe), 3.23 (s, 3H OMe), 3.53 (m, 1H, H-11), 3.65 (s, 3H, ester OMe), 4.23 (m, 1H, H-6), 4.48 (br t, J = 8 Hz, 4H, 2′- and 4′-azetidine CH_2), 4.91 (d, J = 6 Hz, 1H, H-7), 5.15 (d, J = 12 Hz, 1H, H-9), 5.20 (s, 1H, H-19), 5.2–5.35 (br m, 2H, NH_2), 5.47 (br t, J = 9 Hz, 1H, H-5), 6.45 (t, J = 9 Hz, 1H, H-4), 7.35 (d, J = 9 Hz, 1H, H-3); MS *m/z* 640 (M^+ + Na); IR (KBr, cm^{-1}) 1715, 1610, 1525. Anal. ($C_{32}H_{47}N_3O_9$·$0.5H_2O$) C, H, N.

17-(Isopropylamino)-7-oxo-7-decarbamoyl-17-demethoxygeldanamycin (6a). Crude **4d**, prepared from **1d** by the above method[8] (830 mg, 1.52 mmol), was dissolved in 20 mL of chloroform and treated with Dess–Martin reagent (1.30 g, 3.04 mmol) at room temperature with TLC monitoring. After all the **1d** had been consumed (R_f = 0.5 on silica gel eluted with 9:1 methylene chloride:acetone), excess 10% sodium thiosulfate was added and the reaction stirred for 10 min. The mixture was filtered through Celite, and the two layers were separated. The organic layer was dried and evaporated in vacuo to a purple residue which was flash chromatographed on silica gel eluted with 3% acetone in methylene chloride to afford pure **6a**, 430 mg (52%): glass; ^{1}H NMR ($CDCl_3$) δ 0.86 (d, J = 8 Hz, 3H, 14-Me), 1.03 (d, J = 8 Hz, 3H, 10-Me), 1.12 (d, J = 8 Hz, 3H, isopropyl Me), 1.27 (d, J = 8 Hz, 3H, isopropyl Me), 1.45–1.7 (m, 3H, H-13, H-14), 1.80 (s, 3H, 8-Me), 1.95 (s, 3H, 2-Me), 2.03–2.13 (m, 1H, H-15), 2.7–2.8 (m, 2H, H-10, H-15), 3.33 (s, 3H, OMe), 3.35 (s, 3H, OMe), 3.43 (m, 1H, H-12), 3.70 (m, 1H, H-11), 4.05 (br m, J = 8 Hz, 1H, isopropyl CH), 5.07 (d, J = 9 Hz, 1H, H-6), 5.89 (br t, J = 9 Hz, 1H, H-5), 6.10 (d, J = 9 Hz, 1H, NH), 6.57 (t, J = 9 Hz, 1H, H-4), 6.73 (d, J = 12 Hz, 1H, H-9), 6.96 (d, J = 9 Hz, 1H, H-3), 7.10 (s, 1H, H-19), 9.23 (s, 1H, H-22); MS *m/z* 542 (M^+).

17-Azetidinyl-7-decarbamyl-17-demethoxygeldanamycin 7-Oxalate (7a). **4a** (0.250 g, 0.461 mmol) was dissolved in 6 mL of methylene chloride and treated with oxalyl chloride (0.058 g, 0.461 mmol), (dimethylamino)pyridine (0.056 g, 0.461 mmol), and triethylamine (0.093 g, 0.922 mmol) at room temperature for 48 h. The mixture was diluted with 100 mL of methylene chloride, washed with 2 × 50 mL of water and 2 × 50 mL of brine, dried with magnesium sulfate, filtered, and evaporated in vacuo to a purple residue. This was flash chromatographed on silica gel eluted with 3% methanol in chloroform to afford pure **7a**, 0.925 g (34%): mp 152 °C dec; ^{1}H NMR ($CDCl_3$) δ 0.8 (m, 7H, H-13, 14-Me, 10-Me), 1.5 (br m, 2H, H-13, H-14), 1.62 (s, 3H, 8-Me), 1.83 (s, 3H, 2-Me), 2.07 (br m, 1H, H-15), 2.73 (pent, J = 7 Hz, 2H, azetidine CH_2), 2.40 (br d, J = 10 Hz, 1H, H-15), 2.55 (m, 1H, H-10), 3.04 (s, 3H, OMe), 3.16 (s, 3H, OMe), 3.23 (m, 1H, H-12), 3.37 (m, 1H, H-11), 4.15 (d, J = 9 Hz, 1H, H-6), 4.35–4.65 (m, 5H, azetidine 2-CH_2 and 11-OH), 5.13 (s, 1H, H-7), 5.63 (br t, J = 9 Hz, 1H, H-5), 5.77 (d, J = 12 Hz, 1H, H-9), 6.37 (t, J = 9 Hz, 1H, H-4), 6.73 (d, J = 9 Hz, 1H, H-3), 6.92 (s, 1H, H-19), 8.95 (s, 1H, H-22); MS *m/z* 659 (M^+ + 2Na − H); IR (KBr, cm^{-1}) 1765, 1735, 1680, 1645, 1525, 1480. Anal. ($C_{32}H_{42}N_2O_{10}$·$0.5H_2O$) C, H, N.

17-Azetidinyl-11-α-fluorogeldanamycin (8a). Compounds **8a–f** were prepared by the method below. **1a** (0.200 g, 0.342 mmol) was added to a flame-dried flask under nitrogen and dissolved in 15 mL of methylene chloride. The mixture was cooled to −68 °C with an external dry ice/acetone bath, and then a solution of DAST (Aldrich; 0.055 g, 0.342 mmol, 0.045 mL) in 2.5 mL of methylene chloride was added dropwise. After 1 h 5 mL of 5% $NaHCO_3$ was added slowly and

the product extracted into 100 mL of methylene chloride. The organic layer was washed with 3 × 50 mL of water and 2 × 50 mL of brine, dried with $MgSO_4$, filtered, and concentrated to a purple solid. This was purified by flash column chromatography using 5:95 methanol:chloroform. Material of R_f = 0.42 (1:9 methanol:chloroform), desired product (0.058 g, 29%), was dissolved in a minimal amount of ethyl acetate and precipitated with hexane, 0.042 g (21%): mp 128 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.05 (m, 6H, 10-Me, 14-Me), 1.25 (br t, J = 15 Hz, 1H, H-13), 1.55 (br t, J = 15 Hz, 1H, H-13), 1.78 (s, 3H, 8-Me), 1.96 (br m, 1H, H-14), 2.03 (s, 3H, 2-Me), 2.23 (dd, J = 8.5, 16 Hz, 1H, H-15), 2.40 (br m, 2H, 3′-azetidine CH_2), 2.55 (dd, J = 7, 16 Hz, 1H, H-15), 2.80 (br d, J = 26 Hz, 1H, H-10), 3.35 (s, 3H, OMe), 3.37 (s, 3H, OMe), 3.53 (br m, 1H, H-12), 4.39 (d, J = 9 Hz, 1H, H-6), 4.0 (br m, 7H, NH_2, 2′- and 4′-azetidine CH_2, and H-11), 5.70 (s, 1H, H-7), 5.60 (d, J = 9 Hz, 1H, H-9), 5.88 (t, J = 9 Hz, 1H, H-5), 6.55 (t, J = 9 Hz, 1H, H-4), 6.96 (d, J = 9 Hz, 1H, H-3), 7.05 (s, 1H, H-19), 9.33 (s, 1H, NH-22); MS m/z 610 (M^+ + Na); IR (KBr, cm^{-1}) 1735, 1690, 1650. Anal. ($C_{31}H_{42}FN_3O_7$·5H_2O) C, N; H: calcd, 7.73; found, 6.23.

17-(Allylamino)-11-oxogeldanamycin (9a). **1c** (90 mg, 0.15 mmol) was dissolved in $CHCl_3$ (4 mL) to which was added Dess–Martin reagent (382 mg, 0.90 mmol) and the reaction mixture heated to reflux. After 1 h the reaction was complete and the mixture diluted with $CHCl_3$, washed with aqueous sodium thiosulfate and saturated aqueous sodium bicarbonate, and dried over sodium sulfate. The solvent was removed with a rotary evaporator and the residue recrystallized from EtOAc: hexanes to give 17-(allylamino)-11-oxogeldanamycin, 84 mg (96%), as light red crystals: mp 112–118 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.02 (d, 3H, J = 7 Hz), 1.25 (d, 3H, J = 7 Hz), 1.48 (m, 2H), 1.75 (m, 1H), 1.80 (s, 3H), 1.98 (s, 3H), 2.32 (dd, 1H, J = 14, 5 Hz), 2.58 (dd, 1H, J = 14, 7 Hz), 3.29 (overlapping s, 6H), 3.66 (m, 1H), 4.08 (m, 3H), 4.28 (d, 1H, J = 8 Hz), 4.82 (br exchangeable, 2H), 5.18–5.3 (m, 3H), 5.55 (d, 1H, J = 9 Hz), 5.8–6.0 (m, 3H), 6.83 (br exchangeable, 1H), 6.49 (t, 1H, J = 11 Hz), 6.92 (d, 1H, J = 11 Hz), 7.19 (d, 1H), 9.22 (s, 1H); MS m/z 585 (M + 2). Anal. ($C_{31}H_{41}N_3O_8$·0.5EtOAc) C, H, N.

17-Azetidino-11-oximinogeldanamycin (11b). **9b** (0.10 g, 0.17 mmol) was dissolved in ethanol to which was then added a solution of hydroxylamine hydrochloride (0.10 g, 1.42 mmol) and triethylamine (0.2 mL) in ethanol. The reaction mixture was stirred at room temperature for 2.5 h at which time the solvent was removed by rotary evaporation, the residue dissolved in $CHCl_3$, and the organic layer washed with water. After drying over sodium sulfate, the solvent was removed by rotary evaporation and the residue purified by column chromatography (silica gel, 15% acetone:$CHCl_3$) to give 17-azetidino-11-oximinogeldanamycin (70 mg, 68%) as a purple powder: mp 130–145 °C dec; ^{1}H NMR ($CDCl_3$) δ 0.98 (d, 3H, J = 7 Hz), 1.17 (d, 3H, J = 7 Hz), 1.37 (m, 2H), 1.54 (m, 1H), 1.81 (s, 2H), 1.95 (s, 3H), 2.25 (dd, 1H, J = 14, 4 Hz), 2.41 (m, 2H), 2.61 (dd, 1H, J = 14, 3 Hz), 3.20 (s, 3H), 3.28 (s, 3H), 4.02 (m, 2H), 4.13 (d, 1H, J = 8 Hz), 4.65 (t, 1H, J = 8 Hz), 5.01 (br exchangeable, 2H), 5.09 (s, 1H), 5.37 (br d, 1H, J = 10 Hz), 5.74 (t, 1H, J = 10 Hz), 6.45 (t, 1H, J = 12 Hz), 6.89 (br s, 1H), 6.97 (s, 1H), 9.27 (s, 1H); MS m/z 586 (M − 2). Anal. ($C_{31}H_{42}N_4O_8$) H, N, C: calcd, 62.19; found, 61.76.

11-(Allylamino)-17-(methylamino)geldanamycin (12a). In a dry flask, sodium triacetoxyborohydride (152 mg, 0.72 mmol) in dichloroethane (4 mL) was sonicated until a fine suspension was formed. The mixture was removed from the sonicator and treated with allylamine (27 μL, 0.36 mmol) and a few crystals of sodium sulfate. **9c** (100 mg, 0.18 mmol) was then added and the mixture stirred at room temperature for 24 h. The reaction mixture was washed with saturated sodium carbonate and brine and then dried over sodium sulfate. The solvent was removed by rotary evaporation and the crude product purified by column chromatography (silica gel, 9:1 methylene chloride:methanol) to give **12a** as a purple solid, 76 mg (71%): mp 123–126 °C; ^{1}H NMR ($CDCl_3$) δ 0.91 (d, 3H, J = 8 Hz), 0.98 (d, 3H, J = 8 Hz), 1.35 (m, 1H), 1.58 (m, 2H), 1.66 (m, 1H), 1.70 (s, 3H), 1.95 (s, 3H), 2.32 (dd, 1H, J = 8 Hz), 2.75 (dd, 1H, J = 8, 12 Hz), 2.93 (m, 1H), 3.08 (s, 4H), 3.12 (d, 3H, J = 8 Hz), 3.15 (s, 6H), 3.46 (d, 1H, J = 8 Hz), 3.61 (d, 1H, J = 24 Hz), 3.78 (d, 1H, J = 24 Hz), 4.30 (d, 1H, J = 8 Hz), 4.75 (br s, 2H), 5.35 (s, 1H), 5.70 (m, 2H), 6.28 (d, 1H, J = 8 Hz), 6.43 (t, 1H, J = 12 Hz), 7.01 (br d, 1H, J = 16 Hz), 7.08 (s, 1H), 7.20 (m, 5H), 9.42 (s, 1H); MS m/z 649 (M + 1).

11-(Benzylamino)-17-(methylamino)geldanamycin (12b). In the same manner as above, **12b** was prepared from benzylamine and **9c**, 14 mg (48%): mp 123–126 °C; ^{1}H NMR ($CDCl_3$) δ 0.91 (d, 3H, J = 8 Hz), 0.98 (d, 3H, J = 8 Hz), 1.35 (m, 1H), 1.58 (m, 2H), 1.66 (m, 1H), 1.70 (s, 3H), 1.95 (s, 3H), 2.32 (dd, 1H, J = 8 Hz), 2.75 (dd, 1H, J = 8, 12 Hz), 2.93 (m, 1H), 3.08 (s, 4H), 3.12 (d, 3H, J = 8 Hz), 3.15 (s, 6H), 3.46 (d, 1H, J = 8 Hz), 3.61 (d, 1H, J = 24 Hz), 3.78 (d, 1H, J = 24 Hz), 4.30 (d, 1H, J = 8 Hz), 4.75 (br s, 2H), 5.35 (s, 1H), 5.70 (m, 2H), 6.28 (d, 1H, J = 8 Hz), 6.43 (t, 1H, J = 12 Hz), 7.01 (br d, 1H, J = 16 Hz), 7.08 (s, 1H), 7.20 (m, 5H), 9.42 (s, 1H); MS m/z 649 (M + 1).

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