Inhibition of the Oncogene Product p185^{erbB-2} in Vitro and in Vivo by Geldanamycin and Dihydrogeldanamycin Derivatives

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The *erbB-2* oncogene encodes a transmembrane protein tyrosine kinase which plays a pivotal role in signal transduction and has been implicated when overexpressed 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 quinoid moiety 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* (FRE/*erbB-2*). Specifically, dosed intraperitoneally at 100 mg/kg, 17-(allylamino)-17-demethoxygeldanamycin and other 17-amino analogs were effective at reducing p185 phosphotyrosine in subcutaneous flank FRE/*erbB-2* tumors. Modifications to the 17–19-positions of the quinone ring revealed a broad structure–activity relationship in vitro.

Products of cellular proto-oncogenes are highly regulated vital elements of signal transduction, cell growth, and/or development. Mutation or overexpression of proto-oncogenes can perturb regulated mechanisms and trigger uncontrolled cell proliferation. The *erbB-2* oncogene encodes a 185 kDa protein, p185, that has been observed in breast, stomach, and ovarian cancers and appears to be inversely correlated with survival.[1] Specific inhibitors that would block the expression or action of p185 might be useful as anticancer agents. Natural product inhibitors of p185^{erbB-2}, for example, herbimycin A[2] (HBM A) and 4,5-dihydrogeldanamycin[3] (DHGDM), have been reported to cause depletion of the oncoprotein from human breast tumor cells (SKBR-3). Recently, other geldanamycin (GDM) derivatives have been described that cause a depletion of the *erbB-2* gene product from SKBR-3 cells.[4] Geldanamycin analogs have also been found to have antiviral properties, as in the inhibition of cell growth of SV40 virus-infected cells[5] and antitumor activity[5] as well as inhibition of growth of cells transformed by Rauscher leukemia virus.[6] They are reported to inhibit reverse transcriptase, although they exert this effect at concentrations 100–1000 times greater than those needed to inhibit growth of virus-infected cells.[5]

As part of a program aimed at discovering novel, potent, and selective inhibitors of p185^{erbB-2} as useful antitumor agents, we investigated structural modifications of the geldanamycin quinone functionality.[7] This article presents the chemistry, in vitro structure–activity relationships (SAR), and in vivo activity for GDM and DHGDM derivatives modified at their 17–19-positions, i.e., on the quinone ring. An accompanying article describes the chemistry and biological results for other geldanamycins modified along the ansa ring.[8]

Chemistry

Yellow geldanamycin, when treated with alkylamines bearing one displaceable hydrogen, readily reacts at room temperature in chloroform, losing its 17-methoxy group, forming 17-amino-17-demethoxygeldanamycin derivatives **1b1**–**40**[9] as purple solids (see Scheme 1). Similarly, 4,5-dihydrogeldanamycin forms 17-(alkylamino)-17-demethoxy-4,5-dihydrogeldanamycins **1c1**–**c35**, also purple. In some instances where the amine is very bulky or when forcing conditions are employed, the 17-position is not preferentially attacked and instead GDM affords the 19-alkylamino or 17,19-bis(alkylamino) derivatives, **2b1**–**b2** and **3b1**, respectively (Scheme 2). Other known 19-substituted analogs including the 19-bromo compound **4**[10] and geldampicin, **5**,[11] have been prepared and tested for these studies. In using the method of Rinehart for preparation of **5**, we isolated two intermediates, **6** and **7**, from the initial "imine"-forming reaction. The experimental results for **6** were identical to those reported by Rinehart for preparation of his intermediate which he has described as an imine. To this derivative we assigned the different structure shown here for **6** based upon NMR spectra [loss of the 22-NH (at ~9.1 d); COSY and IR, loss of the carbonyl amide (at 1640 cm^{-1})], as well as the mass spectrum. Compound **7**, a minor product in this preparation, was purified by column chromatography and assigned its structure on the basis of high-field ^{13}C and ^{1}H NMR analysis in conjunction with its IR and mass spectrum, which showed an additional CH_2 group.

Modification of the quinone to afford bi- and tricyclic derivatives was pursued. Known geldanoxazinone, **9**,[12] and geldanazine, **10**,[12] were synthesized by the method of Rinehart. The novel 5,6-fused ring system, unknown in quinone ansamycin chemistry, was prepared by treating GDM with a guanidinium salt and potassium *tert*-butoxide in DMF at room temperature, **8b1**–**c4**. These compounds were deep green. In addition, a 7,6-ring iminoquinone, **11**, could be prepared by treatment of GDM with 1,3-diaminopropane followed by dehydration.

The reduction of the quinone moiety of herbimicins **12a,b** and geldanamycin, **13**, has been reported.[13] In the original synthesis by Rinehart of the 18,21-di-

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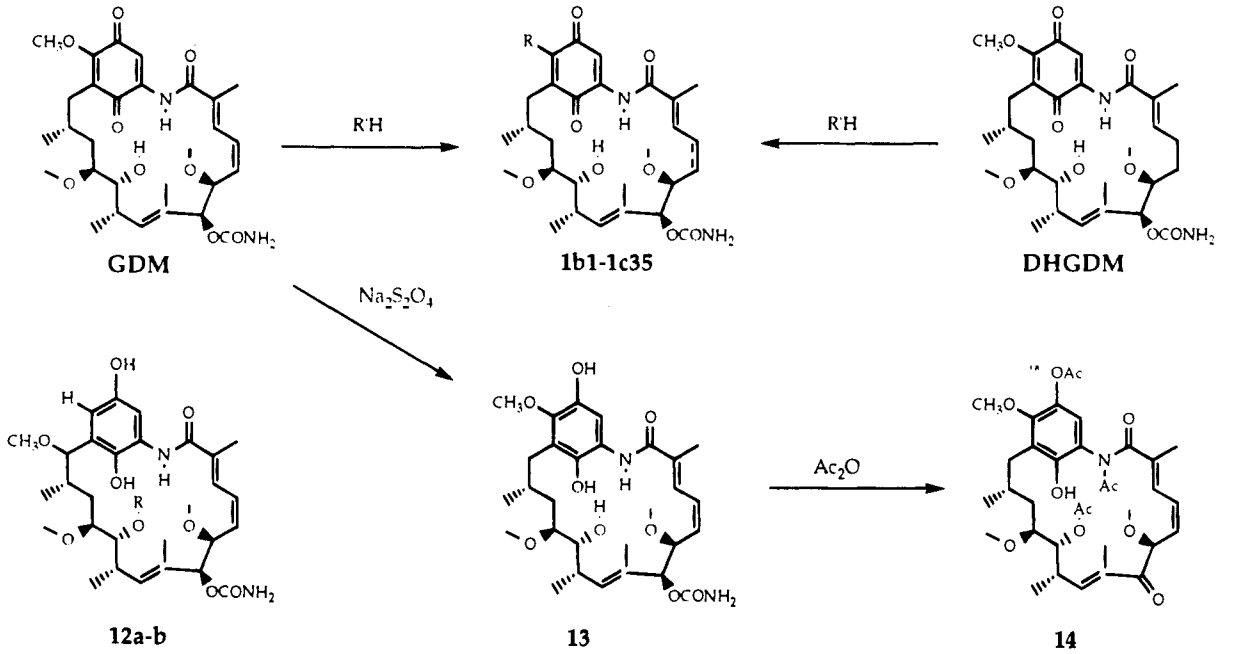
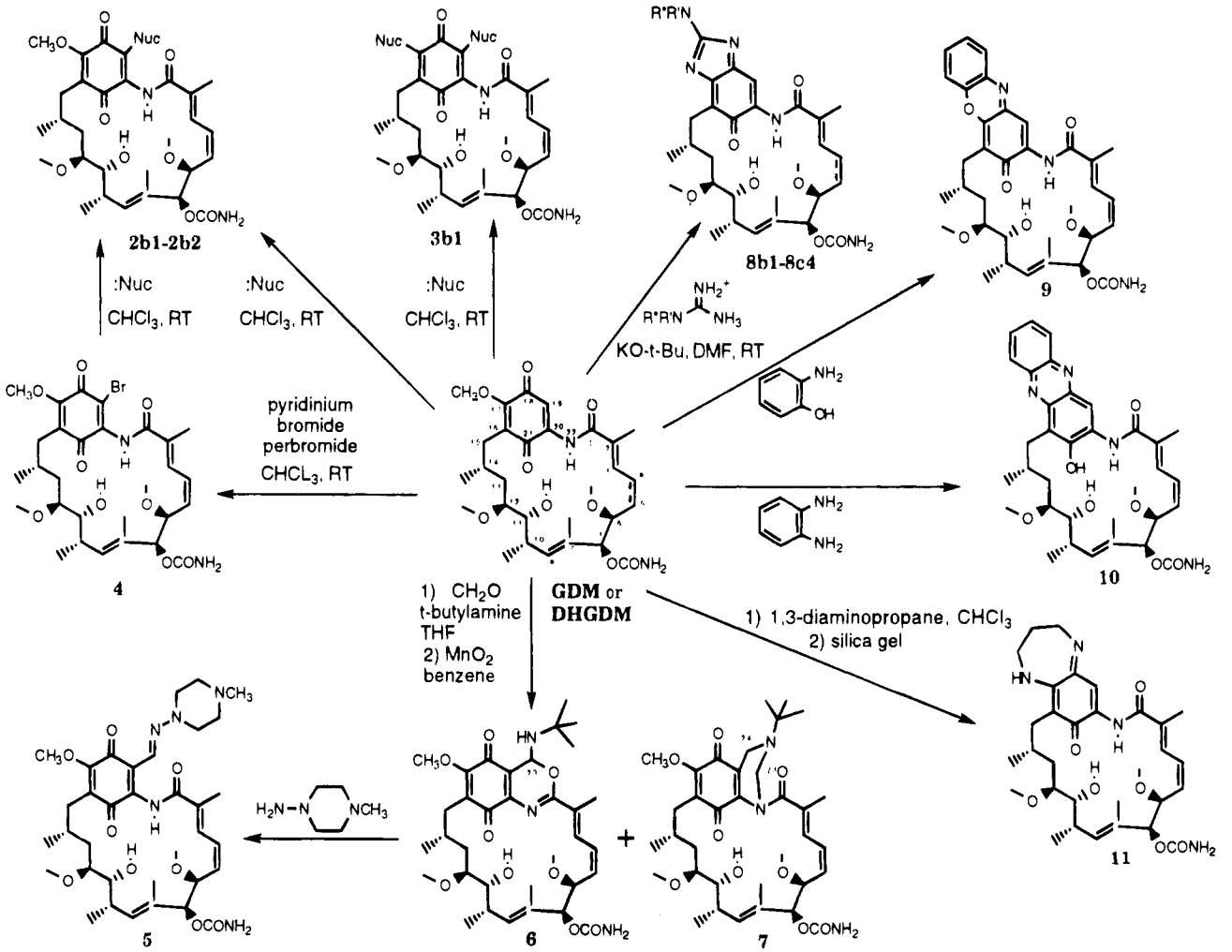
Scheme 1. 17-Aminogeldanamycin Derivatives and Hydroquinones



Scheme 2. Geldanamycin Quinone Modification



acetylgeldanamycin hydroquinone, it was obtained via a zinc acetic anhydride reduction. We obtained free hydroquinone **13** using sodium dithionite. It was stable enough to be isolated, purified for further reactions, and tested in our cellular assay; however, it reverts slowly to the quinone in the presence of air. Treatment of the hydroquinone with acetic anhydride afforded stable triacetate **14** as the major product which was purified by column chromatography. A minor contamination was the tetraacetate, the fourth acetate group being

bound to the 21-position. Reduction of the green imidazoquinone **8b1** with dithionite also proceeded smoothly yielding a faintly yellow product with an NMR spectrum consistent with a hydroquinone-type species (upfield shift of the C-19 proton). However, this material reverted to green quinone overnight in the NMR tube. Similarly, the reduction of a purple 17-amino analog, **1b3**, produced an unstable hydroquinone that was pale yellow and reverted rapidly in air to the purple quinone form. Thus, further chemistries on these unstable hydroquinones were not pursued.

Biology

Inhibition in vitro of $p185^{erbB-2}$ by geldanamycin analogs was determined using an assay which measured depletion of p185 protein upon treatment of SKBR-3 cells with drugs as described by Miller.[4] For the evaluation of ansamycins in vivo, a biochemical end point was measured using a nude mouse model bearing subcutaneous tumors derived from Fisher rat embryo cells that had been transfected with *erbB-2*.[14] Fre/erbB-2 tumors were excised from animals 3 h after either drug or sham treatment (ip) and then pulverized at low temperature and extracted to remove protein. Phosphotyrosine content of $p185^{erb-2}$ in the tumor lysates was evaluated with a sandwich Elisa assay. The p185 was captured with immobilized anti-p185 antibody (Oncogene Sciences Inc.) and the phosphotyrosine content measured with anti-phosphotyrosine antibody.[14] The inhibition of growth of subcutaneous tumors by a geldanamycin derivative was measured in this same system. Drug was dosed ip twice a day for 5 days starting when tumor diameter was 1 cm.

Results and Discussion

Geldanamycin demonstrates moderately potent *erbB-2* inhibition in vitro in SKBR-3 cells and was approximately 4-fold more potent than HBM A and at least 50 times better than DMGDM. This inhibition is revealed as a loss of p185 phosphotyrosine and/or p185 protein as measured by Western blotting. Mechanistic studies on the biological action of ansamycins have been described in detail,[4] showing that they likely do not directly inhibit receptor autophosphorylation since semipurified *erbB-2* was only partially inhibited by HBM A at a dose ~100-fold higher than required for cellular responses.[4] Similarly Miller et al. showed that cellular protein synthesis and viability were not affected by ansamycin treatment for 6 h.[4] Since GDM was ineffective in an in vivo biochemical assay (vide infra), modification of GDM was pursued in order to improve in vitro potency and achieve in vivo activity.

The inhibition data (IC_{50}s) for the series of 17-amino-17-demethoxygeldanamycins and the 17-amino-4,5-dihydro-17-demethoxy geldanamycins are shown in Table 1. Improved potency over GDM was observed with amino and small sterically unconstrained and nonpolar alkylamino groups, e.g., **1b1**–**b10**. Likewise, the same substituents yielded potent DHGDM derivatives, **1c1**–**c16**. The substituents that afforded greatest potency were the (fluoroethyl)amino (**1b1**), IC_{50} = 12 nM, (cyanoethyl)amino (**1b2**), IC_{50} = 17 nM, and azetidinyl (**1b3**), IC_{50} = 23 nM, in the GDM series and methylamino (**1c12**), IC_{50} = 12 nM, and azetidinyl (**1c3**), IC_{50} = 14 nM, in the DHGDM series. Substituents that afforded less active analogs for the GDM series generally corresponded to derivatives with lower potency in the DHGDM analogs, **1c35**,**b35** or **1c23**,**b23**. An exception was **1c10**. Incorporation of acidic functionality in the smaller alkyl groups led to significantly less potent analogs, **1b23**,**b38**. In contrast, basic functions and hydroxy groups in the alkyl moiety were tolerated (**1b11**,**b8**,**b14**,**c8**,**c14**–**c16**). More bulky and aralkylamines afforded very much less active analogs, **1b39**,**b40**,**b27**,**b30**.

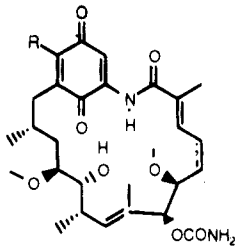
By comparison, nearly all examples of substitution at the 19-position afforded very weakly potent or inactive compounds, **2b1**–**7** (Table 2). This included 17,19-bis(alkylamino) derivatives where the alkylamino group when singly substituted at the 17-position resulted in highly potent inhibition, e.g., **2b2** (IC_{50} > 3250 nM) versus **1b7** (IC_{50} = 43 nM). The potent antibiotic geldampicin, **5**, likewise was inactive against *erbB-2* at concentrations less than 2.9 μM.

Derivatization at the C-18 carbonyl of the quinone supported *erbB-2* inhibition. For example, 17,18-imidazogeldanamycin analogs were active and in some cases equivalent to GDM, **8b1** (IC_{50} = 50 nM; Table 3). This activity was seen in both the GDM and DHGDM series. The potency was in the same range as that seen for the uncyclized guanidine **1b21**. The 7-ring cyclic iminoquinone **11** also retained potency in vitro (IC_{50} = 260 nM). The known cyclic iminoquinone geldanoxazinone, **9**, was 10-fold less potent than the better GDM derivatives, and geldanazine, **10**, was not detectably active.[15]

Hydroquinones of the ansamycins are readily formed and are of varied stability depending upon the substitution of the quinone ring. The amino group is known to destabilize the hydroquinone redox state versus the quinone form.[16] When herbimicin A was incubated in SKBR-3 cell culture and an HPLC analysis of the culture medium was performed, the presence of herbimicin A hydroquinone was detected (data not shown). It is likely that in the in vivo setting both hydroquinone and quinone forms of the drug are accessible via metabolic and/or aerobic interconversion. Table 4 shows the data for a series of hydroquinones. Compound **14** was prepared in an attempt to lock GDM in the hydroquinone state. Our SAR[17] indicates that acetyl groups on the 11- and 22-positions do not decrease biological activity. Since the quinone-type compounds **8b1**, etc., are active, steric bulk and an sp^2 center attached to the 18-position likewise are well tolerated. Thus, the inactivity of **14** along with the greater potency of the 17-aminogeldanamycins corroborates the hypothesis that the action of ansamycins upon *erbB-2* is exerted through its quinone form.

The evaluation of potent GDM analogs in an in vivo animal model was achieved using FRE/*erbB-2* tumors growing subcutaneously in nu/nu mice. These tumors grew to ca. 1 cm in diameter in 12–14 days after subcutaneous implantation of 5×10^5 cells. In contrast, human SKBR-3 tumor cells did not grow satisfactorily as tumor xenographs. Table 5 shows the dose–response effects of herbimicin A, geldanamycin, and compound **1b5** on p185-associated phosphotyrosine levels measured at 6 h after ip dosing. In this test system values >35–40% inhibition were required in order to achieve statistical significance. GDM showed no effect in this assay up to 200 mg/kg and caused lethal toxicity at 400

Table 1. 17-Substituted Geldanamycin and 4,5-Dihydrogeldanamycin Derivatives



1b1-1c35

compd	R	IC_{50}[a,b] (nM)	IC_{50}[c] (nM)	yield[b] (%)	mp[b] (°C)	yield[c] (%)	mp[c] (°C)
GDM	CH_3O	70					
HBM A	H	300					
DMGDM	HO	>3700		*h*			
DHGDM	CH_3O		230				
1b1,c1	F-CH_2CH_2NH	12	27	28	175–176	71	157
1b2,c2	CH-CH_2CH_2NH	17	30	75	151–152	42	130–140
1b3,c3	$-(CH_2)_3N-$	23	14	54	224–225	69	133–134
1b4	$CH{\equiv}CCH_2NH$	26		12	171–172		
1b5,c5	$CH_2{=}CHCH_2NH$	31	51	*f*		55	205
1b6,c6	NH_2	37	40	*g*		75	235–236
1b7,c7	$(CH_2)_2CH$-NH	43	46	*h*	100–104	90	146–147
1b8,c8	$-CH_2CH(OH)CH_2N-$	50	50	72	155[d]	75	
1b9,c9	$(CH_3)_2CHNH$	60	470	81	132–138	71	114
1b10,c10	$CH_3O-CH_2CH_2NH$	66	33	*f*		64	115
1b11	$H_2N-(CH_2)_3NH$	80		40	175–176		
1b12,c12	CH_3NH	89	12	*h*		90	115
1b13,c13	$CH_3S-CH_2CH_2NH$	98	140	43	156–157	55	110
1b14,c14	(*S*)-$CH_3CH(OH)CH_2NH$	99	83	68	143–150[d]	44	133–134
1b15,c15	HO-CH_2CH_2NH	100	34	*h*		54	129
1c16	$-CH_2CH(NH_2)CH_2N-$		66			45	143
1b17	I-$(CH_2)_3NH$	110		53	119–122		
1b18	HO-$(CH_2)_3NH$	130		87	150–156[d]		
1b19	$(CH_3)_2N$-$(CH_2)_3NH$	140		92	178–180		
1b20	HS-CH_2CH_2NH	170		20	156[d]		
1b21	$[(CH_3)_2NH]_2C{=}N$	170		49	140–145		
1b22	(*S*)-HO-$CH_2CH(CH_3)NH$	180		12	148–150		
1b23,c23	COOH-CH_2CH_2NH	190	>3200	84	1430–147	55	117
1b24	$(CH_3)_3CNH$	200		47	102[d]		
1b25	2-thiazoline-NH	220		30	146–148		
1b26	(*R*)-$CH_3CH(OH)CH_2NH$	260		68	141–150[d]		
1b27	furfuryl-CH_2NH	320		78	122–130[d]		
1b28	(*R*)-$HOCH_2CH(CH_3)N$	330		78	143–146		
1b29	$(CH_3O)_2CHCH_2NH$	710		83	179–180		
1b30	benzyl-NH	790		*h*			
1b31	$-CH_2CH_2CH_2(COOH)N-$	790		25	210 d		
1b32	imidazo-$(CH_2)_3NH$	920		84	128–136[d]		
1b33	$HOCH_2CH(OH)CH_2NH$	970		42	140–151[d]		
1b34	CH_3CO-$NHCH_2CH_2NH$	1270		86	130–137[d]		
1b35,c35	(*S*)-$CH_2CH(CH_2OH)CH_2N-$	1300	1300	49	167[d]	44	115
1b36	3-quinuclidine-NH	1370		32	160[d]		
1b37	NH_2COCH_2NH	1990		19	148–150		
1b38[e]	$HOSO_2-CH_2CH_2NH$	>2700		25	152[d]		
1b39	piperidine-4′-CH_2NH	>3110		32	142–160[d]		
1b40	cyclopentyl-NH	>3260		23	165–170[d]		

[a] SKBr-3 cells were incubated at 37 °C for 6 h with a range of various concentrations of geldanamycin analogs in 25% dimethyl sulfoxide (vehicle). Extracts of the cells were separated by electrophoresis. Levels of p185 were measured by immunoblotting with polyclonal antibody NT1 and horseradish peroxidase-conjugated goat anti-mouse IgG. There was no significant change in p185 protein levels in the control samples during this time (ref 4). [b] For geldanamycin analogs. [c] For dihydrogeldanamycin analogs. [d] Decomposed. [e] Triethylamine salt. [f] Ref 19. [g] Ref 6. [h] Ref 5.

mg/kg. While HBM A was significantly active and showed dose–response effects, it illicited a rather weak response. A number of potent alkylamino GDM analogs, e.g., **1b5** (IC_{50} = 31 nM), were more active and demonstrated classical dose–response curves (EC_{50} = 85 mg/kg).

The in vivo inhibitory effects of a series of analogs are shown in Table 6. The top group containing only GDM derivatives is listed in order of decreasing in vitro potency. Within this group the more potent analogs have the better in vivo activity, while compounds with $IC_{50} \geq 50$ nM show lower efficacy or no activity at the highest dose, with the exception of compound **1b14**. The lower portion of the table shows DHGDM analogs which appear generally to be less effective in vivo for a similar in vitro potency, e.g., **1c12,c3** (IC_{50} = 12 and 14 nM, respectively) versus the GDM analogs **1b1,b2** (IC_{50} = 12 and 17 nM, respectively). No DHGDM analog with an in vitro $IC_{50} \geq 35$ nM showed significant in vivo effects.

That the better analogs in the ansamycin series with low nanomolar potencies might translate into

Table 2. 19-Substituted Geldanamycin Derivatives **2–7**

compd	R	IC_{50}[a] (nM)	yield (%)	mp (°C)
2b1	azepine	1500	20	135–137
2b4	cyclopropylamino	>3250	14	g[d]
3b1[b]	17,19-bis(dimethylamino)propylamino	>4100		
6[c]	1-oxo-19-cyclized-*N'*-methyl-*tert*-butylamine	>3100	35	130–135
7[c]	19,22-cyclized-*N',N'*-dimethyl-*tert*-butylamine	>3040	7	130–135
5[c]	$-CH{=}N-N[(CH_2)_2]_2NCH_3$	>2900		
4[b]	bromo	>3130		

[a] Depletion of p185 in SKBR-3 cells.[4] [b] Ref 19. [c] Ref 11. [d] g = glass.

Table 3. 17,18-Cyclized Geldanamycin Derivatives **8b1–11**

compd	nucleophile	IC_{50}[a] (nM)	yield (%)	mp (°C)
GDM		70		
8b1	*N,N*-dimethylguanidine	50	72	231 dec
8c1[b]	*N,N*-dimethylguanidine	50	15	208 dec
8b2	*N,N*-diethylguanidine	210	5	225 dec
8b3	*N*-methyl-*N*-benzylguanidine	300	11	190 dec
8b4	*N*-amidinoazetidine	2500	22	230 dec
8c4[b]	*N*-amidinoazetidine	980	29	190 dec
8b5	*N*-amidino-2'-(hydroxymethyl)azetidine	940	24	214 dec
8b6	*N*-amidino-4'-methylpiperidine	150	38	190 dec
8b7	*N*-amidinomorpholino	2030	19	235 dec
22[c]	o-aminophenol (geldanoxazinone)	420		
10[c]	*o*-phenylenediamine (geldanazine)	>3200		
11	1,3-diaminopropane	260	22	189 dec

[a] Depletion of p185 in SKBR-3 cells.[4] [b] 4,5-Dihydrogeldanamycin backbone. [c] Ref 12.

Table 4. Ansamycin Hydroquinones **12a–14**

backbone	R	IC_{50} (nM)[a]
GDM		70
12a	methyl	520
12b	H	1100
13		100
14	11,18,22-triacyl	>2900

[a] Depletion of p185 in SKBR-3 cells;[4] for comparison, the IC_{50}s for herbimicins A and C quinones **12a,b** are 300 and 270 nM, respectively.

Table 5. In Vivo Dose–Response Inhibition of *erbB-2*

compd	dose ip (mg/kg)	inhibition[a] (%)
herbimicin A		
	50	29
	100	34
	200	49
	400	57
GDM		
	50	30
	100	6
	200	25
	400	lethal
1b5		
	50	27
	100	60
	200	80
	400	72

[a] Decrease in p185 phosphotyrosine from FRE/*erbB-2* tumors 6 h after dosing.[14]

Table 6. In Vivo Structure–Activity Relationships of Geldanamycin Analogs

compd	inhibition (%) 100 mg/kg	inhibition (%) 200 mg/kg	IC_{50} (nM)[b] in vitro
1b1	50	68	12
1b2	na	61	17
1b3	61	68	23
1b5	55	68	31
1b6	45[b]	47	37
8b1	na	nt	50
1b9	na	87	60
1b10	na	na	66
1b14	65	70	99
8b6	na	nt	150
8b4	na	nt	2500
9	na	na	420
1c12	na	37	12
1c14	na	32[c]	14
1c3	38	27	14
1c1	na	53	27
1c10	42	69	33
1c15	na	55	34
1c6	45	44	40
1c7	31	nt	46
1c8	29	44	50
1c5	na	na	51
GDM	na	na	70
HBM A	24	47	300

[a] Mean values are recorded; inhibitions below 25% are recorded as na (not active); nt = not tested. [b] At 50 mg/kg. [c] 66% at 400 mg/kg.

effective inhibitors of *erbB-2* in vivo was not born out by experimentation. None of the structural variations explored resulted in ~100% inhibition at any dose against subcutaneous tumors. Since the in vitro potency was so much greater than the in vivo potency, it is possible that the ansamycin analogs are not distributed efficiently to the tumors, due to rapid metabolism to inactive compounds, rapid excretion, high protein binding, or some other process. Preliminary pharmacokinetic experiments were unable to define more clearly the problem with this poor in vitro to in vivo translation.

It has not been determined what degree of p185 phosphotyrosine inhibition would be required in order to effect tumor growth inhibition in a $p185^{erbB-2}$-driven tumor. Initial experimentation with **1b5** dosed chronically for 5 days twice a day in nu/nu mice bearing flank subcutaneous FRE/*erbB-2* tumors showed a dose–response inhibition of tumor growth with effects at 50 mg/kg reaching statistical significance.[14]

Conclusion

Chemical modification of the geldanamycin and di-

hydrogeldanamycin quinone rings was facile and led to a series of potent inhibitors of the oncogene product p185^{erbB-2}. Optimum substitution at the 17-position of ansamycins identified small alkylamino groups unfunctionalized or bearing hydroxyl or amino groups as the best inhibitors. 17,18-Bicyclic and tricyclic ring quinones were active, similarly. Modifications at the 19-position were not tolerated. GDM derivatives appear to be working through their quinone oxidation state rather than their hydroquinones. Translation from high in vitro potency to good in vivo potency was poor for the GDM series and even less efficient for the DHGDM series. In experiments to be reported elsewhere, compound **1b5** was shown to inhibit the growth of an *erbB-2* dependent tumor in vivo at doses that related to its effect on tumor phosphotyrosine levels as reported in Table 5.[14] Additional results from our research into the SARs of the ansamycins and attempts to identify the molecular targets for ansamycins can be found in the following article.[8]

Experimental Section[18]

17-Azetidin-1-yl-17-demethoxygeldanamycin (1b3). The general procedure for preparing geldanamycin derivatives **1b1**–**b40** found in Table 1 is exemplified by the following. (All *J* values are reported in hertz, Hz.) Geldanamycin (14.0 g, 25.0 mmol) was added to a flame-dried flask under N_2 and slurried in 350 mL of methylene chloride. Azetidine (Aldrich, 2.85 g, 49.9 mmol, 3.36 mL) in 10 mL of methylene chloride was added dropwise. The yellow suspension turned purple during the addition. After 1 h the reaction mixture was evaporated to dryness and the residue dissolved in 50 mL of chloroform and precipitated with 600 mL of hexane. Filtration and vacuum drying at 70 °C afforded pure purple product **1b3**, 14.2 g (97%): mp 225 °C; ^{1}H NMR ($CDCl_3$) δ 0.94 (br t, 6H, 10-Me, 14-Me), 1.2 (m, 1H, H-13), 1.65 (m, 1H, H-13), 1.73 (m, 1H, H-14), 1.76 (s, 3H, 8-Me), 2.0 (s, 3H, 2-Me), 2.17 (dd, J = 12, 16, 1H, H-15), 2.40 (p, J = 8, 2H, 3′-azetidine CH_2), 2.56 (d, J = 16, 1H, H-15), 2.67 (m, 1H, H-10), 3.20 (s, 3H, OMe), 3.30 (s, 3H, OMe), 3.40 (m, 1H, H-12), 3.50 (m, 1H, H-11), 4.25 (d, J = 10.5, 1H, H-6), 4.5–4.10 (m, 6H, 2′- and 4′-azetidine CH_2 and NH_2), 5.13 (s, 1H, H-7), 5.79 (t, J = 9, 1H, H-5), 5.87 (d, J = 9, 1H, H-9), 6.53 (t, J = 9, 1H, H-4), 6.88 (d, J = 9, 1H, H-3), 7.06 (s, 1H, H-19), 10.13 (s, 1H, NH-22); MS *m/z* 608 (M^+ + Na); IR (KBr, cm^{-1}) 1730, 1680, 1645. Anal. ($C_{31}H_{43}N_3O_8$) H, N; C: calcd, 63.54; found, 63.09.

17-(Allylamino)-4,5-dihydro-17-demethoxygeldanamycin (1c5). A general procedure for the preparation of 4,5-dihydrogeldanamycin derivatives **1c1**–**c5** found in Table 1 follows. To 4,5-dihydrogeldanamycin (500 mg, 0.89 mmol) in methylene chloride was added allylamine (402 μL, 5.36 mmol), and the reaction mixture was stirred at room temperature for 24 h at which time TLC analysis indicated the reaction was not complete. The reaction mixture was then heated at reflux for 3 h. The solvent was removed by rotary evaporation and the purple residue partitioned between ethyl acetate and 1 M HCl. The organic layer was dried, the solvent removed by rotary evaporation, and the crude material purified by column chromatography (silica gel, 9:1 methylene chloride/methanol) to give purple 17-(isopropylamino)-4,5-dihydrogeldanamycin **1c5**, 440 mg (84%): mp 114–115 °C; ^{1}H NMR ($CDCl_3$) δ 1.0 (m, 6H, 2 methyl doublets), 1.5–1.8 (m, 8H, contains methyl singlet), 2.10 (s, 3H), 2.2–2.5 (m, 3H), 2.5–2.8 (m, 2H), 3.2–3.5 (m, 8H, contains 2 methyl groups), 3.6 (d, 1H, J = 7), 4.1 (m, 2H), 4.8 (s, 2H), 5.2 (d, 1H, J = 7), 5.25 (d, 1H, J = 10), 5.3 (s, 1H), 5.75 (d, 1H, J = 10), 5.10 (m, 1H), 6.25 (t, 1H, J = 7), 6.4 (br t, 1H), 7.25 (s, 1H), 9.25 (br s, 1H); MS *m/z* 610 (M + Na). Anal. ($C_{31}H_{45}N_3O_8 \cdot 0.5H_2O$) C, H, N.

19-Azepin-1-ylgeldanamycin (2b1). GDM (0.20 g, 0.36 mmol) was dissolved in chloroform and reacted with azepine (0.24 g, 0.36 mmol) at room temperature for 16 h. The mixture was poured into 100 mL of water and extracted with 3 × 100 mL of chloroform. The pooled organic layers were washed with brine, dried with sodium sulfate, filtered, and evaporated in vacuo to a brown/purple solid which was a mixture of derivatives. Preparatory HPLC afforded pure purple **2b1**, 48 mg (20%): t_R 22.26 min (70/30); ^{1}H NMR ($CDCl_3$) δ 0.75 (br d, J = 7, 3H, 14-Me), 0.90 (br d, 3H, J = 7, 10-Me), 1.5–1.8 (br m, 15H, H-13, H-14, 8-Me and 8H from azepin methylenes), 1.94 (s, 3H, 2-Me), 2.30 (dd, J = 8, 16, 1H, H-15), 2.40 (d, J = 16, 1H, H-15), 2.60 (br m, 1H, H-10), 3.20 (s, 3H, OMe), 3.30 (s, 3H, OMe), 3.25–3.40 (br m, 5H, H-12 and 4H from azepine methylenes), 3.50 (m, 1H, H-11), 3.70 (s, 3H, 17-OMe), 4.20 (d, J = 10.5, 1H, H-6), 5.10 (s, 1H, H-7), 5.55–5.75 (br m, 2H, H-5, H-10), 6.42 (t, J = 10, 1H, H-4), 6.85–6.105 (br m, 1H, H-3); MS *m/z* 680 (M^+ + Na); IR (KBr, cm^{-1}) 1725, 1655. Anal. ($C_{35}H_{51}N_3O_9 \cdot H_2O$) C, H, N.

19-(Cyclopropylamino)geldanamycin (2b2). 19-Bromogeldanamycin, **4**[19] (0.23 g, 0.36 mmol), was dissolved in 5 mL of chloroform and treated with cyclopropylamine (152 μL, 2.20 mmol) at 0 °C for 16 h. The mixture was diluted with 100 mL of chloroform, washed with 100 mL of water, dried with sodium sulfate, filtered, and evaporated in vacuo to a dark red residue. Crude **2b2** was purified by column chromatography on silica gel eluted with 15% acetone in hexanes, 30 mg (14%): MS *m/z* 638.3 (M^+ + Na). Anal. ($C_{32}H_{45}N_3O_{10} \cdot 0.25CH_2Cl_2$) C, H, N.

Geldamycin (5) Intermediates 6 and 7. GDM (1.25 g, 2.23 mmol) was dissolved in 25 mL of tetrahydrofuran and treated with *tert*-butylamine (0.49 g, 6.68 mmol) and 37% aqueous formaldehyde (0.14 g, 4.71 mmol) at 45–50 °C for 16 h. The solvent was evaporated in vacuo and residual water azeotroped three times with toluene at aspirator pressure. The residue was dissolved in 25 mL of toluene and 10 mL of chloroform containing manganese dioxide (1.71 g, 19.7 mmol) at 45 °C for 1.5 h. After filtration of the solids, the filtrate was evaporated in vacuo and purified by flash chromatography on silica gel eluted with 10% methanol in chloroform. This purple residue contained **6** and **7**. It was rechromatographed on silica gel eluted with 70% ethyl acetate:29% hexane:1% methanol. Material with R_f = 0.24 was dissolved in minimal ethyl acetate and precipitated with hexanes to afford **6**, 0.50 g (35%), mp 130–135 °C. The structure was assigned by 300 and 500 MHz NMR, COSY, and HetCORR analysis: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.50 (d, J = 7, 3H, 14-Me), 0.65 (m, 1H, H-13), 0.80 (d, J = 7, 3H, 10-Me), 1.15 (m, 1H, H-13), 1.30 (s, 9H, N-*tert*-butyl), 1.32 (s, 3H, 8-Me), 1.75 (m, 1H, H-14), 1.105 (s, 3H, 2-Me), 2.05 (m, 1H, H-10), 2.25 (m, 1H, H-15), 2.35 (m, 1H, H-15), 2.72 (m, 1H, H-12), 2.95 (s, 3H, OMe), 3.12 (s, 3H, OMe), 3.37 (m, 1H, H-11), 3.75 (d, J = 10.5, 1H, H-6), 4.00 (s, 3H, 17-OMe), 4.5 (br s, 2H, NH_2), 4.83 (d, J = 9, 1H, H-7), 5.00–5.15 (m, 2H, H-5, H-10), 6.15–6.30 (m, 2H, H-3, H-4), 8.1–8.25 (br d, J = 9, 10, 1H, H-23), 10.7 and 11.3 (br d, J = 10, 10, 1H, NH); MS *m/z* 646 (M^+); IR (KBr, cm^{-1}) 1730, 1660, 1580. Anal. ($C_{34}H_{50}N_3O_{10} \cdot 0.75H_2O$) C, H, N.

Compound **7**, obtained as a second fraction from the chromatography, had R_f = 0.33, 0.11 g (7%): mp 130–135 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.60 (d, J = 7, 3H, 14-Me), 0.69 (m, 1H, H-13), 1.05 (d, J = 7, 3H, 10-Me), 1.22 (s, 9H, N-*tert*-butyl), 1.37 (s, 3H, 8-Me), 1.59 (m, 1H, H-13), 2.01 (s, 3H, 2-Me), 2.18 (m, 1H, H-14), 2.29 (m, 1H, H-10), 2.50 (d, J = 16, 1H, H-15), 2.60 (d, J = 16, 1H, H-15), 2.87 (m, 1H, H-12), 3.11 (s, 3H, 6-OMe), 3.34 (s, 3H, 12-OMe), 3.42 (d, J = 9, 1H, H-23), 3.61 (d, J = 16, H-24), 3.62 (m, 1H, H-11), 3.88 (d, J = 16, 1H, H-24), 3.99 (t, J = 10, 1H, H-6), 4.06 (s, 3H, 17-OMe), 4.15 (br s, 2H, NH_2), 5.02 (d, J = 9, 1H, H-7), 5.23 (m, 2H, H-5, H-10), 5.38 (d, J = 9, 1H, H-23), 6.34 (t, J = 9, 1H, H-4), 6.43 (d, J = 9, 1H, H-3); IR (KBr, cm^{-1}) 1730, 1670, 1640, 1580; MS *m/z* 658 (M^+). Anal. ($C_{35}H_{52}N_3O_9 \cdot H_2O$) C, H, N.

24-(Dimethylamino)-17,18-imidazo-17-demethoxygeldanamycin (8b1). A general procedure for making GDM and DHGDM derivatives **8b1**–**c4** starting with the appropriate geldanamycin starting material follows. 1,1-Dimethylguanidinium sulfate (Aldrich; 0.7314 g, 2.676 mmol) was ground to a fine powder with mortar and pestle, added to a flame-dried flask under nitrogen, and slurried in 10 mL of dimethylformamide. Potassium *tert*-butoxide (Aldrich; 0.600 g, 5.35 mmol) was added, and the reaction mixture was stirred

at room temperature for 10 min. Geldanamycin (0.500 g, 0.892 mmol) was added, and the reaction mixture immediately turned purple. The reaction mixture was stirred overnight, and the color changed from purple to dark green. The reaction mixture was diluted with 100 mL of ethyl acetate and washed with 0.36 mL of acetic acid in 50 mL of water followed by brine (3 × 50 mL) and water (2 × 50 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated to dryness. The green residue was purified by flash column chromatography on 400 g of silica gel eluted with 3% methanol chloroform to afford a green residue which was dissolved in a minimal amount of chloroform, precipitated with hexane, filtered, and dried in vacuo. **8b1**: 0.384 g (72%); mp 231 °C dec; ^{1}H NMR ($CDCl_3$) δ 0.86 (d, J = 5, 6H, 10-Me, 14-Me), 1.47–1.81 (br m, 3H, H-13, H-14), 1.72 (s, 3H, 8-Me), 1.96 (s, 3H, 2-Me), 2.24–2.46 (br m, 2H, H-15), 2.70 (m, 1H, H-10), 3.21 (s, 3H, OMe), 3.25 (s, 3H, OMe), 3.32 (s, 3H, 24-N-Me), 3.35–3.52 (m, 1H, H-12), 3.42 (s, 3H, 24-N-Me), 3.55 (m, 1H, H-11), 4.25 (d, J = 9, 1H, H-6), 4.56–4.71 (br m, 2H, NH_2), 5.10 (s, 1H, H-7), 5.76 (br m, 2H, H-5, H-9), 6.50 (t, J = 9, 1H, H-4), 6.85 (d, J = 9, 1H, H-3), 7.48 (s, 1H, H-19), 8.92 (s, 1H, H-22); MS m/z 622.3 (M^+ + Na); IR ($CHCl_3$, cm^{-1}) 1730, 1675, 1580. Anal. ($C_{31}H_{43}N_5O_7 \cdot 0.5H_2O$) C. Calcd: H, 7.30; N, 11.55. Found: H, 6.45; N, 10.62.

17,18-Diazepino-17-demethoxygeldanamycin (11). Geldanamycin (200 mg, 0.36 mmol) was reacted with 1,3-diaminopropane (184 μL, 2.20 mmol) in 5 mL of chloroform for 30 min at room temperature to give the mono-17-substituted intermediate which was worked up to afford 217 mg of crude residue by the method of **1b3**. This was dissolved in a slurry of 5 mL of chloroform and 2 mL of methanol containing 1.5 g of silica gel. After 4 h at room temperature, a new peak was observed by TLC. The mixture was filtered, evaporated in vacuo to a residue, column chromatographed on silica gel eluted with 3% methanol in chloroform, and then rechromatographed on silica gel with 20% acetone in chloroform. **11**: 46 mg (22%); ^{1}H NMR ($CDCl_3$) δ 1.0–1.1 (m, 7H, H-13, 10-Me, 14-Me), 1.8–1.9 (br s, 4H, H-13, 8-Me), 1.95–2.1 (br m, 2H, H-14, H-15), 2.10 (s, 3H, 2-Me), 2.4–2.5 (br m, 2H, diazepine CH_2), 2.25–2.4 (m, 2H, H-10, H-15), 3.2–3.3 (m, 2H, diazepine CH_2), 3.32 (s, 3H, OMe), 3.42 (s, 3H, OMe), 3.50–3.55 (m, 1H, H-12), 3.62–3.72 (m, 1H, H-11), 3.95–4.15 (m, 2H, diazepine CH_2), 4.37 (d, J = 9, 1H, H-6), 4.8–5.1 (br m, 2H, NH_2), 5.20 (s, 1H, H-7), 5.47 (br t, 1H, diazepine CH_2), 5.83 (t, J = 9, 1H, H-5), 6.00 (d, J = 9, 1H, H-9), 6.62 (t, J = 9, 1H, H-4), 6.95 (d, J = 9, 1H, H-3), 7.75 (s, 1H, H-19), 8.95 (s, 1H, H-22); MS m/z 585.4 (M^+). Anal. C, H, N.

11,18,22-Triacetylgeldanamycin Hydroquinone (14). Geldanamycin (1.00 g, 1.78 mmol) was dissolved in 100 mL of ethyl acetate and stirred with a 10% solution of sodium dithionite (72 mL). After 0.5 h the organic layer was washed with 3 × 50 mL of water, dried with magnesium sulfate, filtered under a nitrogen atmosphere, and evaporated in vacuo to a pale yellow residue. This was dissolved in a minimal amount of chloroform and precipitated with hexanes. **13**: 0.604 g (60%); ^{1}H NMR ($CDCl_3$) δ 0.86 (m, 6H, 10-Me, 14-Me), 1.05–1.15 (br s, 2H, H-13), 1.45 (s, 3H, 8-Me), 1.60 (s, 3H, 2-Me), 2.15 (br d, J = 16, 1H, H-15), 2.55–2.70 (m, 2H, H-10, H-15), 3.05 (s, 3H, OMe), 3.18 (s, 3H, OMe), 3.28 (m, 1H, H-12), 3.47 (m, 1H, H-11), 3.65 (s, 3H, 17-OMe), 4.14 (d, J = 9, 1H, H-6), 4.78 (s, 1H, H-7), 5.44 (t, J = 9, 1H, H-5), 5.80 (br d, J = 9, 1H, H-9), 6.20 (t, J = 9, 1H, H-4), 6.58 (br d, J = 9, 1H, H-3), 7.10 (s, 1H, H-19), 7.85 (br s, 1H, OH), 7.95 (br s, 1H, OH), 8.15 (s, 1H, H-22).

This material was used immediately in the next step. Thus, compound **13** (0.20 g, 0.36 mmol) was slurried in 6 mL of methylene chloride and treated with (dimethylamino)pyridine (0.13 g, 1.07 mmol), triethylamine (0.22 g, 2.13 mmol), and then acetic anhydride (0.18 g, 1.78 mmol) at room temperature. After 16 h the mixture was poured into 100 mL of chloroform and washed with 3 × 50 mL of brine and 3 × 50 mL of water, dried with magnesium sulfate, filtered, and evaporated in vacuo to a pale yellow residue. This was column chromatographed on silica gel eluted with 10% methanol in chloroform to give 11 mg of R_f = 0.53 material (NMR is consistent with a tetraacetyl compound) and triacetate **14**, 89 mg (36%): ^{1}H NMR ($CDCl_3$) δ 0.70 (d, J = 7, 3H, 10-Me), 0.85 (d, J = 7, 3H, 14-Me), 1.35–1.5 (m, 2H, H-13), 1.60 (s, 3H, 8-Me), 2.05 (br s, 10H, H-15, acetyl Me), 2.25 (s, 3H, 2-Me), 2.5–2.7 (m, 2H, H-15, H-10), 3.08 (br s, 4H, H-12, OMe), 3.26 (s, 3H, OMe), 3.70 (s, 3H, 17-OMe), 4.13 (br t, J = 7, 1H, H-6), 4.5–4.8 (m, 2H, NH_2), 4.100 (d, J = 7, 1H, H-11), 5.08 (d, J = 7, 1H, H-7), 5.15 (d, J = 9, 1H, H-10), 5.74 (t, J = 9, 1H, H-5), 6.58 (t, J = 9, 1H, H-4), 6.74 (br, 1H, H-3), 7.45–7.7 (br s, 1H, OH), 7.62 (s, 1H, H-19); MS m/z 711 (M^+ + Na); IR (KBr, cm^{-1}) 1725, 1595. Anal. ($C_{35}H_{47}N_2O_{11} \cdot H_2O$) C, H, N.

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