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Bioorganic & Medicinal Chemistry 12 (2004) 5317–5329
Bioorganic & Medicinal Chemistry

Synthesis and biological activities of novel 17-aminogeldanamycin derivatives

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Received 14 April 2004; revised 19 May 2004; accepted 24 July 2004
Available online 3 September 2004

Abstract—Geldanamycin interferes with the action of heat shock protein 90 (Hsp90) by binding to the *N*-terminal ATP binding site and inhibiting an essential ATPase activity. In a program directed toward finding potent, water soluble inhibitors of Hsp90, we prepared a library of over sixty 17-alkylamino-17-demethoxygeldanamycin analogs, and compared their affinity for Hsp90, ability to inhibit growth of SKBr3 mammalian cells, and in selected cases, water solubility. Over 20 analogs showed cell growth inhibition potencies similar to that of 17-allylamino-17-demethoxygeldanamycin (17-AAG), the front-runner geldanamycin analog that is currently in multiple clinical trials. Many of these analogs showed water solubility properties that were desirable for formulation. One of the most potent and water-soluble analogs in the series was 17-(2-dimethylaminoethyl)amino-17-demethoxygeldanamycin (17-DMAG), which was independently prepared by the NCI and will soon enter clinical trials. Importantly, the binding affinity of these analogs to the molecular target Hsp90 does not correlate well with their cytotoxicity in SKBr3 cells.

1. Introduction

Hsp90 is an essential protein that chaperones multiple growth-regulatory signaling proteins, referred to as 'client proteins'.[1,2] Geldanamycin (Fig. 1A), a benzoquinone ansamycin antibiotic,[3,4] binds to the *N*-terminal domain ATP binding site of Hsp90, inhibiting the chaperone activity of the protein and leading to disruption of the Hsp90-client protein complexes.[5–7] 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, geldanamycin and its analogs have potential utility in cancer chemotherapy.[8,9]

Geldanamycin is a potent cytotoxic agent,[10–12] but shows significant hepatotoxicity in animals.[13] One of the potential causes of this toxicity is the C-17 methoxy group, which is reactive toward nucleophiles commonly found in biological molecules. Analogs of geldanamycin with alkylamino groups in place of the methoxy moiety at C-17 (Fig. 1B) are less reactive toward nucleophiles and have excellent biological activity and reduced hepatotoxicity.[14,15] The tolerance at the 17-position for diverse substituents is consistent with structures of the complex of Hsp90 and geldanamycin or its analogs

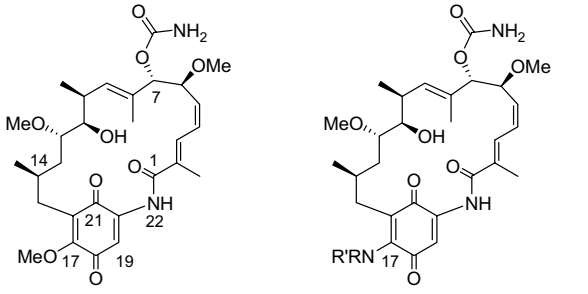

Figure 1. Structure drawing and atom numbering of geldanamycin derivatives.

Keywords: Hsp90; Geldanamycin; 17-Alkylaminogeldanamycin; 17-DMAG; 17-AAG; Anti-cancer.
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doi:10.1016/j.bmc.2004.07.053

showing that the 17-position of geldanamycin is exposed to solvent.[16,17] Of the numerous 17-alkylamino-17-demethoxygeldanamycin analogs that have been prepared, 17-AAG (NSC330507) demonstrated sufficient antitumor activity in vivo to warrant introduction in clinical trials.[18–20] Despite promising antitumor properties, 17-AAG's low solubility in water makes it difficult to formulate.

Most of the 17-alkylamino-17-demethoxygeldanamycin analogs previously disclosed in the literature were prepared before Hsp90 had been identified as the molecular target of the geldanamycins. Consequently few studies were sufficiently extensive to permit the development of structure–activity relationships. In the present work, we prepared a library of more than sixty 17-alkylamino-17-demethoxygeldanamycin analogs and determined their binding affinities to recombinant Hsp90, growth inhibition of SKBr3 cells, and in relevant cases, water solubility. From this study, numerous potent 17-alkylamino-17-demethoxygeldanamycins were identified, several of which are sufficiently soluble in water to warrant more extensive evaluation. It is of interest that the most attractive analog uncovered in our study was 17-DMAG. Independently, the NCI demonstrated the efficacy, water solubility, and oral bioavailability of 17-DMAG (NSC 707545), and selected it for advanced studies.[21,22]

2. Synthesis and evaluation of compounds

Three classes of side chains for the 17-amino group were used: (a) homologous series (e.g., methyl, ethyl, propyl) to elucidate the effect of size of the 17-side chain on activities; (b) amines with side chains bearing hydrophilic groups such as hydroxyl and amino groups; and (c) side chains that possess specific structural features including enhanced rigidity that might improve binding affinity.

17-Amino derivatives of geldanamycin are generally obtained in good yields by treating geldanamycin (GDM) with primary or small ring cyclic amines in a variety of solvents. More sterically hindered secondary amines react with geldanamycin to give the 19-amino derivatives as the predominant product.

For example, whereas *N*,*N*-dimethylethylenediamine reacts at the 17-position with GDM to give 17-DMAG, *N*,*N*,*N*′-trimethylethylenediamine reacts with GDM to give exclusively 19-(*N*-(2-dimethylaminoethyl)-*N*-methyl)aminogeldanamycin. It is plausible that a small amine adds to both the C-17 and the C-19-positions of the quinone ring. While the C-19-adduct may revert back to GDM, the irreversible loss of MeOH from the C-17-adduct results in the 17-amino-17-demethoxygeldanamycin derivatives (**1**). When the amine nucleophile is bulky, steric hindrance caused by the methoxy group prevents addition to the 17-position. The C-19-adduct may slowly tautomerize to give the hydroquinone intermediate, which is oxidized to the quinone form (**2**) upon exposure to air.[23]

The biological activities of the analogs synthesized were evaluated using tumor cell growth inhibition assays and Hsp90 binding assays. The human breast tumor SKBr3 cell line[24,25] was used as the primary cell line for screening. Cell viability was determined using the CellTiter-Glo™ Luminescent Cell Viability Assay.[26] We devised a Scintillation Proximity Assay (SPA)[27] to measure binding to Hsp90 as described in the experimental section, which has a higher throughput than a previously described filter binding assay.[28] Binding constants for a group of 22 analogs were measured using both the *N*-terminal domain and the full-length protein of human Hsp90. Although good correlation ($R = 0.8$) was observed between the two assays, only the binding affinity to full-length recombinant Hsp90 is reported. The solubility of potent analogs was evaluated in aqueous phosphate buffer at pH 7.

3. Results and discussion

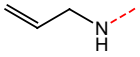
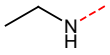
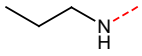
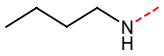
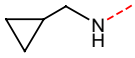
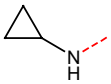
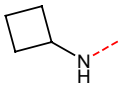
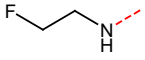
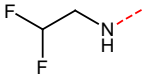
3.1. SAR for cytotoxicity

Analogs synthesized from simple C_0–C_4 alkyl primary amines are listed in Table 1. A two-carbon unit appears to provide the optimal chain length for cytotoxicity in SKBr3 cells. Bulky chains (butyl and cyclobutyl) show reduced cytotoxicity, although results for derivatives from bulkier amines (Table 2) show that the reduction is limited for side chains that are unbranched near C-17. Hsp90 binding affinity for these compounds all fell within a factor of two of one another, with the exception of 17-amino-17-demethoxygeldanamycin (**3**).

One of the objectives of this investigation was to identify geldanamycin analogs with better water solubility than 17-AAG. We synthesized a series of derivatives from commercially available hydroxylalkylamines (Table 3). SKBr3 cell growth inhibition activities of analogs from 2-hydroxyethylamine derivatives (compounds **19**–**23**) again showed that larger sized side chains tend to decrease activity. The variation of activities among the four diastereomers (compounds **24**–**27**) from 2-(hydroxymethyl)cyclohexylamine suggests that the stereochemistry in the side chain does have an impact on the cytotoxicity, while Hsp90 binding is not affected. The analog from 6-amino-6-deoxyglucose (**29**) showed improved potency for Hsp90 binding, although the cytotoxicity was lost.

Water solubility could also be enhanced by the introduction of charged functionality at C-17, including amino groups, heterocycles, and carboxy groups (Table 4). The 2-carboxyethylamino derivative (**39**) was inactive in the SKBr3 cell growth inhibition assay. This result is consistent with the weak activity of a sulfonic acid derivative reported by Schnur et al.[15,29] Analogs with heterocycles (compounds **34**–**38**) showed modest potency in SKBr3 cell growth inhibition. Among the derivatives from diamines, a two-carbon spacer between the nitrogen atoms appeared to be optimal for cytotoxicity. Compound **30**, 17-(2-dimethylaminoethyl)amino-17-demethoxygeldanamycin (17-DMAG) is over 10-fold

Table 1. Structures and biological activities for 17-aminogeldanamycin derivatives from simple alkylamines

Compound	R_1R_2N–	IC_{50} (nM)[a]	K_d (μM)[b]
17-AAG[c]		33 ± 10	1.3
3[c]		33	0.1
4[c]		92	0.8
5[d]		18 ± 2	1.4
6[d]		42 ± 24	2.6
7		380 ± 5	3.3
8		39 ± 17	2.1
9[c]		27	1.3
10		270 ± 110	2.1
11[c]		17 ± 3	0.8
12		19	0.8

[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 the full-length human Hsp90.

[c] Similar IC_{50} values were reported for inhibition of $p185^{erbB-2}$ by Schnur et al.[15]

[d] Compound was reported by Sasaki and Inoue.[14] The biological activity in SKBr3 cells was not reported.

more potent than 17-(4-dimethylaminobutyl)amino-17-demethoxygeldanamycin (**32**). As was seen in other series, these analogs showed similar binding affinities in the Hsp90 binding assay.

We expanded the series with a two-carbon spacer and investigated ethylenediamine derivatives with systematic variations (Table 5). Addition of methyl groups to each position resulted in decreased SKBr3 cell growth inhibition activities. Substitution at the α-position (**40**) produced a marked decrease of activity compared to β-substitution (**41**). Replacing the terminal dimethylamino group with cyclic amines resulted in analogs with a range of activities. The aziridinyl analog (**46**) gave the highest level of cytotoxicity, followed by the azetidinyl analog (**47**), which is similar in cytotoxicity to 17-DMAG. Analogs with larger rings showed decreased cytotoxicity. Efforts to synthesize the *N*-demethyl and *N*,*N*-di-demethyl analogs of 17-DMAG from *N*-methylethylenediamine and ethylenediamine, respectively, did not give the desired products. Although disclosed by Sasaki and Inoue,[14] these analogs appear to be unstable, undergoing a cyclization reaction via reaction of the terminal amine with the 18-carbonyl group. The *N*-acetyl version (**53**) of the di-demethyl analog showed significantly reduced cytotoxicity, perhaps due to the reduced basicity of the nitrogen or greater bulk. The terminal nitrogen could be oxidized to the *N*-oxide (**54**) or converted to a quaternary ammonium salt (**55**). Both compounds lost cytotoxic activities while maintaining the Hsp90 binding activities.

We also investigated the impact that changes in the basicity of the amine (Table 1) or diamine (Table 5) might have on cytotoxicity by introducing fluoro substituents on the alkyl chain. The pK_a's of protonated forms of ethylamine, 2-fluoroethylamine, and 2,2-difluoroethylamine are 10.6, 9.0, and 7.5, respectively.[30,31] However, geldanamycin derivatives from these amines (**5**, **11**, and **12**) show equal potency in SKBr3 cell growth inhibition assays. Modifying the basicity of the remote nitrogen in the ethylenediamine series by introducing fluorine substituents (compounds **42**–**44**) had no impact on the cytotoxicity of these analogs, although decreasing basicity of this atom appears to decrease the Hsp90 binding affinity modestly.

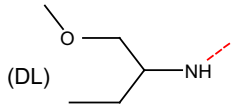
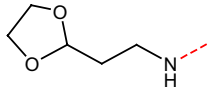
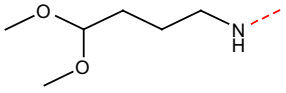
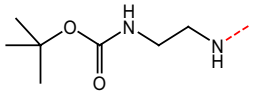
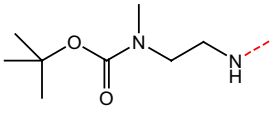
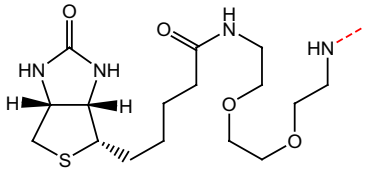
Cyclic amines containing three, four, or five atoms react with geldanamycin to give the 17-derivatives (Table 6). The SKBr3 cell growth inhibition activities are lower for these compounds than for 17-AAG, with the exception of 17-azetidinyl-17-demethoxygeldanamycin (**60**). Introducing a dimethylamino substituent on the aziridine derivative (**58** and **59**) appeared to improve the activities modestly. However, the substitution on the azetidine analog (**61**) decreased the cytotoxicity.

3.2. Hsp90 binding activity

A co-crystal structure of 17-DMAG with human Hsp90 reveals that it binds to Hsp90 in a similar fashion as GDM.[17] The 17-(2-dimethylaminoethyl) side chain is solvent exposed, which is consistent with the similar Hsp90 binding activities observed for most of the 17-alkylamino-17-demethoxygeldanamcyin analogs. In the exceptional cases of compounds **3** and **29**, extra H-bonds are possible with the 17-NH_2 group or the OH groups in the sugar moiety, respectively.

Surprisingly, there was a large discrepancy between the Hsp90 binding assay and the tumor cell growth inhibition. Whereas the IC_{50} values for cytotoxicity vary over 200-fold, the K_d values for binding to Hsp90 of most of these analogs lie within a narrow range (ca. 6-fold). This discrepancy may be due to one or more reasons. First, the compounds must cross the cell membrane to reach the target Hsp90. The inactivity of compounds **18**, **29**, **39**, **54**, and **55** may be due to their inability to cross the cell membrane because of polar or charged groups. Second, for compounds that cross the cell membrane,

Table 2. Structures and biological activities of 17-aminogeldanamycin derivatives from bulky alkylamines

Compound	R_1R_2N–	IC_{50} (nM)	K_d (μM)
13[a]		620	1.9
14		62 ± 3	1.6
15		180	2.4
16		170	1.3
17		320	0.6
18		>1000	1.3

[a] A racemic mixture of the amine was used in the synthesis. An equal mixture of diastereomers were obtained and tested.

Table 3. Structures and biological activities of 17-aminogeldanamycin derivatives from hydroxyalkylamines

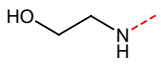
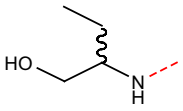
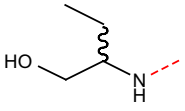
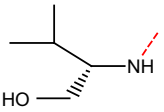
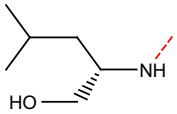
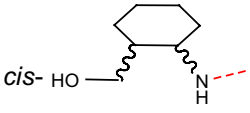
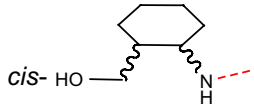
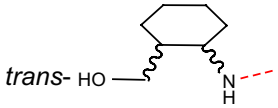
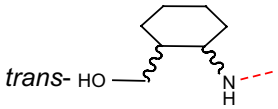
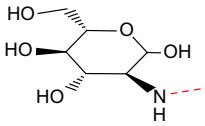
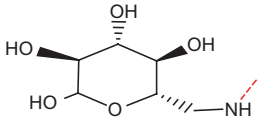
Compound	R_1R_2N–	IC_{50} (nM)	K_d (μM)
19[a]		50	0.8
20[b]		200	0.8
21[c]		45	1.3
22		640	2.5
23		1500	2.1
24[b]		1700	2.4

Table 3 (*continued*)

Compound	R_1R_2N–	IC_{50} (nM)	K_d (μM)
25[c]		600	2.1
26[b]		180	1.9
27[c]		850	3.2
28		1200	0.5
29		5300	0.2

[a] Similar IC_{50} values were reported for inhibition of $p185^{erbB-2}$ by Schnur et al.[15]
[b] More polar diastereomer (eluted earlier on reversed-phase HPLC) isolated from the mixture obtained using a racemic mixture of the amine.
[c] Less polar diastereomer (eluted later on reversed-phase HPLC) isolated from the mixture obtained using a racemic mixture of the amine.

the level of intra-cellular accumulation may differ. It has been observed that geldanamycin accumulates in cells to achieve a cytosolic concentration that is 100-fold greater than the extra-cellular concentration.[32,33] Further, we have recently shown that several analogs reported here that have similar affinity to Hsp90 are concentrated in cells in proportion to their cytotoxicity (unpublished results). Finally, the binding affinity of the Hsp90 complex in tumor cells for geldanamycin analogs may be different from that measured using purified Hsp90. Kamal et al. reported over 100-fold difference in binding affinity of 17-AAG to the Hsp90 complex derived from tumor cells and normal cells,[34] and the magnitude of differential binding may be sensitive to the structure of the 17-substituents. Regardless of the mechanism, it is clear that a meaningful SAR cannot be developed by analysis of ligand binding to Hsp90.

3.3. Water solubility

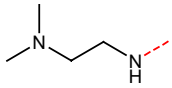
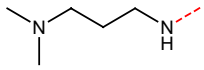
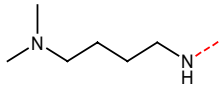
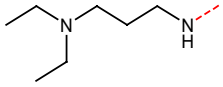
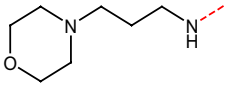
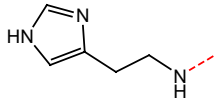
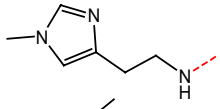
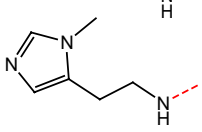
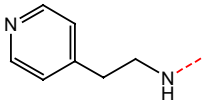
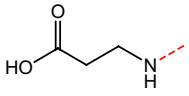
We measured the water solubility of a series of analogs, most of which have IC_{50} below 100 nM. Saturated solutions of GDM analogs in pH 7 phosphate buffer were obtained following 1 day of dissolution, with no further increase in concentration observed following the additional incubation. The concentrations of saturated solutions of selected analogs are shown in Table 7. About 15 analogs showed over a 5-fold increase in solubility relative to 17-AAG.

4. Conclusion

A systematic investigation of the C-17-side chains in 17-amino-17-demethoxygeldanamycin analogs resulted in a number of compounds with desirable properties for further evaluation. We found over 20 analogs that showed SKBr3 cell growth inhibition IC_{50} below 100 nM. At least ten of these potent analogs are more soluble than 17-AAG by at least 5-fold in pH 7 phosphate buffer. Analogs with small linear side chains at the 17-position tend to give better cytotoxic activities than ones with bulkier and branched 17-side chains. Fluoro, hydroxyl, or amino substitution is well tolerated. The basicity of the amines did not show relationship to the cytotoxicity. The SAR showed that the binding affinity to Hsp90 is not significantly affected by substituents at the 17-position, which makes this position useful for tuning physical and pharmacological properties for geldanamycin analogs.

An important general finding of this work is that the in vitro binding of geldanamycin analogs to purified Hsp90 bears little relationship to the cytotoxicity of the analog. This observation could be related to differences in intracellular concentrations for the various analogs. Thus, the binding affinity measured for geldanamycin analogs to the molecular target Hsp90 is of limited utility for discovery or optimization of new geldanamycin-based cytotoxic agents. Indeed, cellular uptake of

Table 4. Structures and biological activities of 17-aminogeldanamycin derivatives with charged 17-side chains

Compound	R_1R_2N–	IC_{50} (nM)	K_d (μM)
30		24 ± 8	0.5
31[a]		88	0.6
32		350 ± 40	0.9
33		250	0.7
34		120	0.9
35		98	0.8
36		58 ± 25	1.1
37		140	0.9
38		180	1.3
39		>5000	1.1

[a] Compound was previously reported by Schnur et al.[15]

analogs that meet criterion of sufficient Hsp90 binding affinity may be the more important parameter to optimize.[32]

5. Experimental

5.1. Chemistry

5.1.1. General experimental methods. Unless noted otherwise, all reagents and solvents were purchased from commercial sources and used without purification. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in $CDCl_3$ solution with a Bruker DRX 400 spectrometer. Chemical shifts were referenced to δ 7.26 and 77.0 ppm for ^{1}H 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. Flash chromatography was performed on silica gel, eluted with 1–5% methanol in dichloromethane. 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).

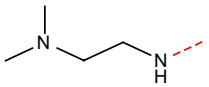
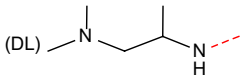
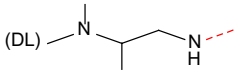
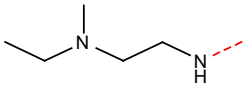
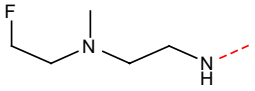
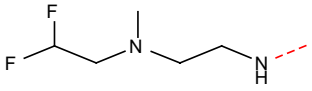
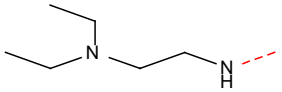
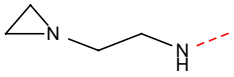
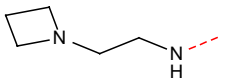
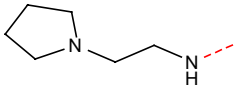
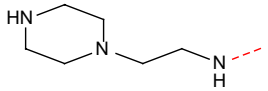
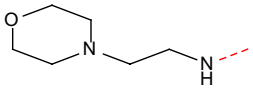
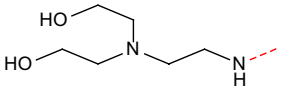
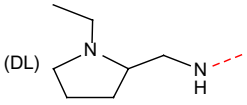
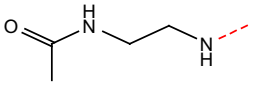
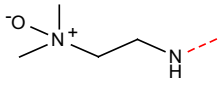
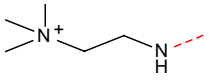
5.1.2. Synthetic procedures. 17-Alkylamino-17-demethyoxygeldanamycin compounds were synthesized from geldanamycin and the corresponding alkylamine using Method A if the amine is in the free base form or Method B if the amine is in the hydrochloride salt form.

5.1.2.1. Method A. To a solution of geldanamycin (56 mg, 0.1 mmol) in 1,2-dichloroethane (4 mL) was added the amine (0.2 mmol). After the mixture was stirred at 20 °C for 20 h, it was diluted in ethyl acetate and washed with aqueous bicarbonate and brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The crude product was purified either by flash chromatography or by reversed-phase HPLC, giving the product as a purple solid.

5.1.2.2. Method B. To a solution of geldanamycin (56 mg, 0.1 mmol) in a mixture of 1,2-dichloroethane (3 mL) and methanol (1 mL) was added the amine hydrochloride salt (0.2 mmol) and triethylamine (0.3 mmol). The mixture was stirred at 50 °C until the starting material geldanamycin was fully consumed by TLC. The reaction was worked up as in Method A, giving the product as a purple solid.

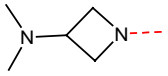
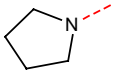
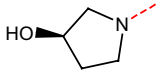
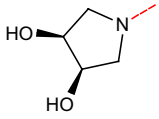
5.1.3. 17-[2-(Dimethylamino)ethyl]amino-17-demethyoxygeldanamycin (30). To a solution of geldanamycin (56 mg, 0.1 mmol) in 1,2-dichloroethane (4 mL) was added *N*,*N*-dimethylethylenediamine (22 μL, 0.2 mmol). After stirred at 20 °C for 20 h, the mixture was diluted in ethyl acetate and washed with aqueous bicarbonate and brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The crude product was purified by flash chromatography on silica gel, eluted with 1–5% methanol in dichloromethane, giving 59 mg of 17-DMAG as a purple solid. ^{1}H NMR: δ 0.95 (d, 3H, J = 6.8 Hz), 0.97 (d, 3H, J = 6.8 Hz), 1.71–1.78 (m, 3H), 1.78 (s, 3H), 2.00 (s, 3H), 2.25 (s, 6H), 2.38 (dd, 1H, J = 11.2, 13.6 Hz), 2.55 (t, 2H, J = 5.8 Hz), 2.66 (d, 1H, J = 14.0 Hz), 2.72 (m, 1H), 3.24 (s, 3H), 3.34 (s, 3H), 3.42–3.47 (m, 2H), 3.55 (m, 1H), 3.66 (m, 1H), 4.29 (d, 1H, J = 10.0 Hz), 4.45 (br s, 1H), 5.02 (br s, 2H), 5.16 (s, 1H), 5.83 (t, 1H, J = 10.4 Hz), 5.89 (d, 1H, J = 9.2 Hz), 6.56 (t, 1H, J = 11.4 Hz), 6.94 (d, 1H, J = 11.6 Hz), 7.04 (br s, 1H), 7.23 (s, 1H), 9.17 (s, 1H). ^{13}C NMR: δ 12.2, 12.5, 12.7, 22.9, 28.4, 32.2, 34.3, 35.0, 42.6, 44.7, 56.6, 57.0, 57.1, 72.5, 81.2, 81.4, 81.5, 108.6, 108.8, 126.5, 126.8, 132.7, 133.8, 134.9, 135.6, 141.1, 145.4, 156.2, 168.3, 180.0, 184.0. ESI-TOF-MS *m/z* 617.3527, calcd for $C_{32}H_{49}N_4O_8$ ($[M+H]^+$) 617.3545.

Table 5. Structures and biological activities of 17-aminogeldanamycin derivatives from substituted ethylenediamines

Compound	R_1R_2N–	IC_{50} (nM)	K_d (μM)
30		24 ± 8	0.5
40[a]		130	0.7
41[a]		50 ± 20	0.4
42		97	0.55
43		77	1.0
44		85	1.3
45		91 ± 3	0.5
46		16	3.0
47		26	0.44
48		70 ± 2	0.4
49		470	1.2
50		140	0.5
51		380	0.8
52[a]		560	1.1
53		520	0.9
54		>1000	0.6
55		>1000	0.3

[a] A racemic mixture of the amine was used in the synthesis. An equal mixture of diastereomers were obtained and tested.

Table 6. Structures and biological activities of 17-aminogeldanamycin derivatives from cyclic amines

Compound	R_1R_2N–	IC_{50} (nM)	K_d (μM)
57[a]		500	1.6
58[c]		270	1.4
59[b]		200	0.9
60		24 ± 5	1.4
61		180 ± 60	1.5
62		450	0.8
63		310	0.5
64		840	0.3

[a] A racemic mixture of the amine was used in the synthesis. An equal mixture of diastereomers were obtained and tested.
[b] More polar diastereomer (eluted earlier on reversed-phase HPLC) isolated from the mixture obtained using a racemic mixture of the amine.
[c] Less polar diastereomer (eluted later on reversed-phase HPLC) isolated from the mixture obtained using a racemic mixture of the amine.

5.1.4. [2-(17-Demethoxygeldanamycin-17-ylamino)-ethyl]-dimethylamine *N*-oxide (54). To a solution of 17-DMAG (18 mg, 30 μmol) in dichloromethane (1 mL) was added 3-chloroperbenzoic acid (77% max, 12 mg, 50 μmol max). The mixture was stirred at 20 °C for 20 h. LC/MS showed that the reaction was complete. The crude product was purified by HPLC on a C18 column, eluted using a gradient of acetonitrile in water. The product was obtained as a purple solid, 7 mg. ^{1}H NMR: δ 0.96 (d, 3, J = 6.8 Hz), 0.98 (d, 3, J = 8.8 Hz), 1.71–1.78 (m, 3), 1.78 (s, 3), 2.00 (s, 3), 2.18 (dd, 1, J = 10.4, 11.2 Hz), 2.57 (d, 1, J = 14.0 Hz), 2.72 (m, 1), 2.24 (s, 3), 3.32 (s, 3), 3.33 (s, 6), 3.40 (m, 1), 3.48–3.58 (m, 3), 4.24 (m, 2), 4.29 (d, 1, J = 10.4 Hz), 5.06 (br s, 2), 5.15 (s, 1), 5.83 (t, 1, J = 10.0 Hz), 5.91 (d, 1, J = 12.0 Hz), 6.56 (t, 1, J = 12.0 Hz), 6.95 (d, 1, J = 12.0 Hz), 7.12 (s, 1), 9.15 (s, 1), 10.06 (br s, 1). ^{13}C NMR: δ 12.1, 12.5, 12.7, 23.0, 28.6, 32.2, 34.4, 34.9, 42.7, 56.6, 57.0, 59.1, 66.7, 72.3, 81.3, 81.5, 81.7, 109.2, 111.7, 126.6, 126.8, 132.6, 134.0, 135.0, 135.6, 140.4, 146.3, 156.2, 168.4, 178.7, 186.1. ESI-TOF-MS m/z 633.3507, calcd for $C_{32}H_{49}N_4O_9$ ($[M+H]^+$) 633.3494.

5.1.5. 17-Butylamino-17-demethoxygeldanamycin (7). ^{1}H NMR: δ 0.95–1.02 (m, 9), 1.41–1.5 0 (m, 2), 1.62–1.81 (m, 5), 1.79 (s, 3), 2.02 (s, 3), 2.41–2.46 (m, 1), 2.67 (d, 1, J = 14.0 Hz), 3.74 (t, 1, J = 7.6 Hz), 3.26 (s, 3), 3.36 (s, 3), 3.41–3.46 (m, 2), 3.48–3.58 (m, 2), 4.30 (d, 1, J = 9.6 Hz), 4.85 (s, br, 2), 5.18 (s, 1), 5.85 (t, 1, J = 10.4 Hz), 5.89 (d, 1, J = 10.4 Hz), 6.26–6.29 (m, 1), 6.58 (t, 1, J = 11.2 Hz), 6.95 (d, 1, J = 11.6 Hz), 7.27 (s, 1), 9.19 (s, 1). ^{13}C NMR: δ 12.4, 12.6, 12.8, 13.7, 19.9, 22.9, 28.5, 31.8, 32.3, 34.4, 35.1, 45.6, 56.7, 57.1, 72.6, 81.2, 81.5, 81.7, 108.3, 108.6, 126.5, 126.9, 132.7, 133.8, 135.0, 135.8, 141.5, 144.9, 156.1, 168.4, 180.6, 183.9. ESI-TOF-MS m/z 509.2996, calcd for $C_{30}H_{41}N_2O_5$ ($[M-H_2NCO_2-CH_3OH]^+$)[35] 509.3010.

Table 7. Solubility of selected analogs in aqueous solutions[a]

Compound	Solubility (mg/mL)
17-AAG	0.10 ± 0.01
3	0.2
5	0.6
6	0.6
8	0.5
9	0.12 ± 0.08
11	0.3
14	0.14 0.07
19	0.6
30	1.4 ± 0.1
31	4.6
32	2.4 ± 0.1
34	0.8
39	11
41	2.5 ± 0.6
42	2.7 ± 0.4
43	0.4
44	0.13 ± 0.06
45	1
46	0.3 ± 0.1
47	0.6
48	3.7
49	1.5 ± 0.2
60	0.4

[a] Measured in 50 mM sodium phosphate buffer at pH 7.0.

5.1.6. 17-(Cyclopropylmethyl)amino-17-demethoxygeldanamycin (8). ^{1}H NMR: δ 0.31 (dd, 2, J = 5.2, 10.8 Hz), 0.67 (dd, 2, J = 5.6, 13.2 Hz), 0.95 (d, 3, J = 6.4 Hz), 0.97 (d, 3, J = 6.8 Hz), 1.05–1.12 (m, 1), 1.65–1.85 (m, 3), 1.79 (s, 3), 2.02 (s, 3), 2.38 (dd, 1, J = 9.6, 14 Hz), 2.65 (d, 1, J = 13.6 Hz), 2.66–2.75 (m, 1), 3.25 (s, 3), 3.26–3.5 0 (m, 3), 3.55–3.59 (m, 1), 3.36 (s, 3), 4.31 (d, 1, J = 10.0 Hz), 4.71–4.82 (m, 2), 5.18 (s, 1), 5.81–5.93 (m, 2), 6.41 (t, 1, J = 4.2 Hz), 6.58 (t, 1, J = 11.6 Hz), 6.95 (d, 1, J = 11.6 Hz), 7.28 (s, 1), 9.18 (s, 1). ^{13}C NMR: δ 3.8, 3.9, 11.1, 12.4, 12.6, 12.8, 22.9, 28.5, 32.3, 34.4, 35.1, 51.2, 56.7, 57.1, 72.6, 81.2, 81.5, 81.7, 108.4, 108.7, 126.5, 126.9, 132.7, 133.8, 135.0, 135.8, 141.4, 144.8, 156.0, 168.4, 180.6, 183.8. ESI-TOF-MS m/z 507.2861, calcd for $C_{30}H_{49}N_2O_5$ ($[M-H_2NCO_2-CH_3OH]^+$) 507.2853.

5.1.7. 17-Cyclobutylamino-17-demethoxygeldanamycin (10). ^{1}H NMR: δ 0.94 (d, 3, J = 6.4 Hz), 0.98 (d, 3, J = 6.8 Hz), 1.71–2.07 (m, 7), 1.78 (s, 3), 2.01 (s, 3), 2.23 (dd, 1, J = 10.4, 13.6 Hz), 2.32–2.36 (m, 1), 2.48–2.63 (m, 1), 2.62 (d, 1, J = 13.2 Hz), 2.73 (m, 1), 3.25 (s, 3), 3.35 (s, 3), 3.41–3.45 (m, 1), 3.55 (t, 1, J = 6.4 Hz), 4.2 0 (s, br, 1), 4.23 (d, 1, J = 9.6 Hz),

4.31–4.36 (m, 1), 4.94 (br s, 2), 5.16 (s, 1), 5.84 (t, 1, $J = 10.4$ Hz), 5.88 (d, 1, $J = 7.6$ Hz), 6.45 (d, 1, $J = 6.8$ Hz), 6.56 (t, 1, $J = 11.6$ Hz), 6.94 (d, 1, $J = 11.6$ Hz), 7.25 (s, 1), 9.15 (s, 1). ^{13}C NMR: δ 12.3, 12.6, 12.8, 14.7, 23.0, 28.5, 31.2, 32.3, 33.0, 34.4, 35.0, 49.5, 56.7, 57.1, 72.6, 81.3, 81.5, 81.6, 108.5, 108.7, 126.5, 126.9, 132.8, 133.7, 134.9, 135.8, 141.3, 143.9, 156.1, 168.3, 180.6, 183.9. ESI-TOF-MS *m/z* 507.2879, calcd for $C_{30}H_{39}N_2O_5$ ($[M-H_2NCO_2-CH_3OH]^+$) 507.2854.

5.1.8. 17-[2-(Dioxolan-2-yl)ethyl]amino-17-demethoxygeldanamycin (14). ^{1}H NMR: δ 0.95 (d, 3, $J = 7.6$ Hz), 0.97 (d, 3, $J = 7.2$ Hz), 1.61–1.77 (m, 3), 1.77 (s, 3), 2.01 (s, 3), 2.06 (dd, 2, $J = 6.4$, 10.4 Hz), 2.41 (dd, 1, $J = 10.8$, 13.6 Hz), 2.65 (d, 1, $J = 14.4$ Hz), 2.66–2.74 (m, 1), 3.23 (s, 3), 3.33 (s, 3), 3.42 (d, 1, $J = 8.8$ Hz), 3.53–3.63 (m, 2), 3.74–3.78 (m, 1), 3.86–3.89 (m, 2), 3.97–4.01 (m, 2), 4.28 (d, 1, $J = 10.0$ Hz), 4.33 (s, br, 1), 5.01 (t, 1, $J = 4.0$ Hz), 5.02 (s, br, 2), 5.15 (s, 1), 5.83 (t, 1, $J = 10.8$ Hz), 5.87 (d, 1, $J = 9.6$ Hz), 6.55 (t, 1, $J = 11.2$ Hz), 6.86–6.96 (m, 2), 7.22 (s, 1), 9.16 (s, 1). ^{13}C NMR: δ 12.3, 12.6, 12.8, 22.8, 28.5, 32.3, 32.5, 34.4, 35.1, 41.1, 56.7, 57.0, 65.1, 72.6, 81.2, 81.5, 81.6, 102.7, 108.4, 108.7, 126.5, 126.9, 132.7, 133.7, 135.0, 135.7, 141.3, 145.3, 156.2, 168.4, 180.4, 184.0. ESI-TOF-MS *m/z* 553.2880, calcd for $C_{31}H_{41}N_2O_7$ ($[M-H_2NCO_2-CH_3OH]^+$) 553.2908.

5.1.9. 17-(4,4-Dimethoxybutyl)amino-17-demethoxygeldanamycin (15). ^{1}H NMR: δ 0.95 (d, 3, $J = 6.4$ Hz), 0.98 (d, 3, $J = 6.8$ Hz), 1.71–1.8 0 (m, 7), 1.78 (s, 3), 2.05 (s, 3), 2.40 (t, 1, $J = 10.8$ Hz), 2.66 (d, 1, $J = 14.0$ Hz), 2.68–2.75 (m, 1), 3.25 (s, 3), 3.33 (s, 6), 3.35 (s, 3), 3.3–3.6 (m, 4), 4.29 (d, 1, $J = 10.0$ Hz), 4.37 (t, 1, $J = 4.8$ Hz), 4.94 (s, br, 2), 5.17 (s, 1), 5.84 (t, 1, $J = 10.4$ Hz), 5.88 (d, 1), 6.37 (t, 1, $J = 5.2$ Hz), 6.57 (t, 1, $J = 11.6$ Hz), 6.94 (d, 1, $J = 11.6$ Hz), 7.25 (s, 1), 9.17 (s, 1). ^{13}C NMR: δ 12.4, 12.6, 12.8, 22.9, 24.8, 28.5, 29.8, 32.3, 34.4, 35.1, 45.6, 53.2, 56.7, 57.1, 72.6, 81.2, 81.5, 81.6, 104.0, 108.4, 108.7, 126.5, 126.9, 132.8, 133.7, 135.0, 135.8, 141.4, 144.9, 156.1, 168.4, 180.6, 183.9. ESI TOF MS *m/z* 569.3309, calcd for $C_{32}H_{45}N_2O_7$ ($[M-H_2NCO_2-CH_3OH]^+$) 569.3221.

5.1.10. Biotinyl-linker-AG (18). ^{1}H NMR: δ 0.96 (s, 3), 0.98 (s, 3), 1.42 (m, 2), 1.61–1.69 (m, 4), 1.71–1.78 (m, 3), 1.78 (s, 3), 2.01 (s, 3), 2.22 (t, 2, $J = 7.4$ Hz), 2.42 (m, 1), 2.66 (d, 1, $J = 17$ Hz), 2.71 (m, 1), 2.73 (m, 1), 2.87 (dd, 1, $J = 4.8$, 12.8 Hz), 3.13 (m, 1), 3.27 (s, 3), 3.35 (s, 3), 3.38–3.50 (m, 3), 3.45–3.79 (m, 12), 4.31 (d, 1, $J = 9.6$ Hz), 4.27 (m, 1), 4.46 (m, 1), 5.10 (br s, 2), 5.18 (s, 1), 5.44 (s, 1), 5.86 (t, 1, $J = 11.2$ Hz), 5.87 (d, 1, $J = 10.0$ Hz), 6.17 (s, 1), 6.57 (t, 1, $J = 11.2$ Hz), 6.76 (m, 2), 6.95 (d, 1, $J = 11.2$ Hz), 7.24 (s, 1), 9.21 (s, 1). ^{13}C NMR: δ 12.4, 12.6, 12.8, 23.0, 25.5, 28.0, 28.1, 29.7, 32.3, 34.2, 34.8, 35.9, 39.2, 40.5, 45.2, 55.3, 56.7, 57.2, 60.1, 61.7, 68.6, 70.0, 70.2, 70.4, 72.6, 81.3, 81.4, 81.6, 108.5, 108.8, 126.4, 127.2, 132.8, 133.5, 134.7, 136.2, 141.5, 145.0, 156.3, 163.6, 168.5, 173.4, 180.5, 184.2. ESI-TOF-MS *m/z* 903.4555, calcd for $C_{44}H_{67}N_6O_{12}S$ ($[M+H]^+$) 903.4532.

5.1.11. 17-[(2*S*)-1-Hydroxy-3-methylbut-2-yl]amino-17-demethoxygeldanamycin (22). ^{1}H NMR: δ 0.97–1.17 (m, 12), 1.63–1.71 (m, 3), 1.79 (s, 3), 1.91–2.03 (m, 1), 2.05 (s, 3), 2.26–2.32 (m, 1), 2.66 (d, 1, $J = 13.6$ Hz), 2.71–2.77 (m, 1), 3.26 (s, 3), 3.35 (s, 3), 3.41–3.46 (m, 2), 3.52–3.58 (m, 1), 3.72 (d, 2, $J = 4.4$ Hz), 3.88–3.94 (m, 1), 4.21–4.25 (m, 1), 4.31 (d, 1, $J = 10.0$ Hz), 4.83 (s, br, 2), 5.86 (t, 1, $J = 10.8$ Hz), 5.88 (d, 1, $J = 8.8$ Hz), 6.37 (d, 1, $J = 9.6$ Hz), 6.58 (t, 1, $J = 11.6$ Hz), 6.94 (d, 1, $J = 12.0$ Hz), 7.27 (s, 1), 9.15 (s, 1). ^{13}C NMR: δ 12.4, 12.6, 12.8, 18.6, 19.4, 23.1, 28.8, 29.8, 32.3, 34.5, 35.0, 56.7, 57.1, 60.5, 62.4, 72.6, 81.2, 81.4, 81.7, 108.7, 108.9, 126.6, 127.0, 132.8, 133.7, 135.0, 135.8, 141.2, 145.3, 156.1, 168.3, 180.5, 184.1. ESI-TOF-MS *m/z* 539.3125, calcd for $C_{31}H_{43}N_2O_6$ ($[M-H_2NCO_2-CH_3OH]^+$) 539.3116.

5.1.12. 17-[(2*S*)-1-Hydroxy-4-methylpent-2-yl]amino-17-demethoxygeldanamycin (23). ^{1}H NMR: δ 0.87–1.01 (m, 12), 1.45–1.51 (m, 1), 1.64–1.93 (m, 5), 1.78 (s, 3), 2.03 (s, 3), 2.48 (dd, 1, $J = 10.8$, 14 Hz), 2.67 (d, 1, $J = 14.0$ Hz), 2.71–2.76 (m, 1), 3.26 (s, 3), 3.32 (s, 3), 3.35–3.44 (m, 1), 3.55–3.63 (m, 3), 4.11–4.17 (m, 1), 4.24–4.29 (m, 1), 4.29 (d, 1, $J = 9.6$ Hz), 4.93 (s, br, 2), 5.16 (s, 1), 5.85 (t, 1, $J = 10.8$ Hz), 5.86 (d, 1, $J = 10.4$ Hz), 6.19 (d, 1, $J = 8.8$ Hz), 6.57 (t, 1, $J = 11.6$ Hz), 6.93 (d, 1, $J = 11.6$ Hz), 7.24 (s, 1), 9.12 (s, 1). ^{13}C NMR: δ 12.4, 12.6, 12.8, 22.6, 23.0, 23.2, 25.0, 28.7, 32.3, 34.8, 35.0, 41.7, 53.8, 56.7, 57.1, 64.1, 72.6, 81.2, 81.4, 81.7, 108.9, 109.0, 126.6, 127.0, 132.8, 133.7, 135.0, 135.9, 141.1, 144.9, 156.2, 168.4, 180.5, 184.0. ESI-TOF-MS *m/z* 553.3263, calcd for $C_{32}H_{45}N_2O_6$ ($[M-H_2NCO_2-CH_3OH]^+$) 553.3272.

5.1.13. 17-[2-(Hydroxymethyl)cyclohexyl]amino-17-demethoxygeldanamycin, the less polar diastereomer from racemic *cis*-1-amino-2-(hydroxymethyl)cyclohexane (25). ^{1}H NMR: δ 0.97 (d, 3, $J = 6.4$ Hz), 0.99 (d, 3, $J = 6.8$ Hz), 1.35–1.95 (m, 12), 1.79 (s, 3), 2.02 (s, 3), 2.15–2.23 (m, 1), 2.61–2.70 (m, 1), 3.25 (s, 3), 3.35 (s, 3), 3.44 (d, 1, $J = 9.2$ Hz), 3.58–3.63 (m, 2), 3.75 (t, 1, $J = 9.6$ Hz), 4.30 (d, 1, $J = 10.0$ Hz), 4.40 (s, br, 2), 4.87 (s, br, 1), 5.17 (s, 1), 5.85 (t, 1, $J = 10.8$ Hz), 5.90 (d, 1, $J = 5.2$ Hz), 6.58 (t, 1, $J = 11.2$ Hz), 6.95 (d, 1, $J = 11.6$ Hz), 7.10 (d, 1, $J = 8.0$ Hz), 9.19 (s, 1). ^{13}C NMR: δ 12.3, 12.6, 12.8, 21.1, 22.8, 24.1, 24.3, 28.8, 29.6, 32.3, 34.7, 35.1, 41.8, 50.9, 56.7, 57.1, 64.3, 72.6, 81.2, 81.5, 81.7, 108.7, 109.0, 126.6, 126.9, 132.8, 133.8, 135.0, 135.7, 141.4, 144.4, 156.1, 168.4, 180.3, 184.3. ESI-TOF-MS *m/z* 565.3302, calcd for $C_{33}H_{45}N_2O_6$ ($[M-H_2NCO_2-CH_3OH]^+$) 565.3272.

5.1.14. 17-(Glucos-2-amino)-17-demethoxygeldanamycin (28). ^{1}H NMR (CD_3OD): δ (relative to CHD_2OD at 3.30 ppm) 0.97 (d, 3, $J = 7.2$ Hz), 1.00 (d, 3, $J = 7.2$ Hz), 1.55–1.85 (m, 3), 1.73 (s, 3), 2.00 (s, 3), 2.48 (dd, 1, $J = 8.8$, 13.2 Hz), 2.71–2.80 (m, 2), 3.28 (s, 3), 3.25–3.95 (m, 7), 3.34 (s, 3), 4.18 (dd, 1, $J = 9.6$, 2.8 Hz), 4.53 (d, 1, $J = 8.0$ Hz), 5.15 (d, 1, $J = 3.2$ Hz), 5.22 (br s, 1), 5.61 (d, 1, $J = 9.2$ Hz), 5.85 (t, 1, $J = 9.6$ Hz), 6.61 (t, 1, $J = 11.4$ Hz), 7.06 (s, 1), 7.14 (d, 1, $J = 11.6$ Hz). ^{13}C NMR (CD_3OD): δ (relative to CD_3OD at 49.0 ppm) 12.4, 13.6, 14.4, 22.6, 31.5, 33.7,

34.6, 36.0, 56.9, 57.5, 62.6, 71.9, 73.3, 74.0, 74.9, 81.8 (2C), 81.8, 82.7, 92.8, 109.4, 110.9, 127.1, 129.6, 132.4, 134.5, 135.4, 137.7, 142.4, 147.5, 159.0, 170.7, 181.3, 185.8. ESI TOF MS *m/z* 730.3204, calcd for $C_{34}H_{49}N_3O_{13}Na$ ($[M+Na]^+$) 730.3158.

5.1.15. 17-(Glucos-6-amino)-17-demethoxygeldanamycin (29). ^1H NMR (CD_3OD): δ (relative to CHD_2OD at 3.30 ppm) 0.97 (d, 3, $J = 6.8$ Hz), 1.00 (d, 3, $J = 6.4$ Hz), 1.55–1.85 (m, 3), 1.73 (s, 3), 1.99 (s, 3), 2.41 (dd, 1, $J = 9.6$, 13.6 Hz), 2.65–2.80 (m, 2), 3.29 (s, 3), 3.15–4.05 (m, 7), 3.34 (s, 3), 3.57 (m, 1), 4.52 (d, 1, $J = 8.4$ Hz), 4.55 (d, 0.5, $J = 7.6$ Hz), 5.16 (d, 0.5, $J = 3.6$ Hz), 5.19 (br s, 1), 5.60 (d, 1, $J = 9.6$ Hz), 5.87 (t, 1, $J = 9.6$ Hz), 6.61 (t, 1, $J = 11.4$ Hz), 7.05 (s, 1), 7.11 (d, 1, $J = 11.6$ Hz). ^{13}C NMR (CD_3OD): δ (relative to CD_3OD at 49.0 ppm) 12.4, 13.6, 14.3, 22.7, 31.5, 33.6, 34.5, 35.7, 56.8, 57.5, 70.7 (2C), 73.4, 73.8 (2C), 74.3 (2C), 74.6, 75.4, 76.2, 77.7, 82.0 (2C), 83.0, 94.1, 98.4, 109.1, 110.0, 127.1, 129.6, 132.6, 134.4, 135.3, 138.0, 142.7, 146.7, 159.1, 170.6, 181.2, 185.6. ESI-TOF-MS *m/z* 730.3156, calcd for $C_{34}H_{49}N_3O_{13}Na$ ($[M+Na]^+$) 730.3158.

5.1.16. 17-[4-(Dimethylamino)butyl]amino-17-demethoxygeldanamycin (32). ^1H NMR: δ 0.95 (d, 3, $J = 6.4$ Hz), 0.98 (d, 3, $J = 7.2$ Hz), 1.61–1.75 (m, 7), 1.78 (s, 3), 2.01 (s, 3), 2.30 (s, 6), 2.38–2.45 (m, 3), 2.61–2.75 (m, 2), 3.43–3.65 (s, 3, $J = 14.0$ Hz), 3.35 (s, 3), 3.43–3.65 (m, 4), 4.29 (d, 1, $J = 10.0$ Hz), 4.92 (br s, 2), 5.16 (s, 1), 5.84 (t, 1, $J = 10.8$ Hz), 5.89 (d, 1, $J = 12.0$ Hz), 6.51 (br s, 1), 6.57 (t, 1, $J = 11.8$ Hz), 6.94 (d, 1, $J = 11.6$ Hz), 7.25 (s, 1), 9.16 (s, 1). ^{13}C NMR: δ 12.3, 12.6, 12.7, 22.9, 24.4, 27.5, 28.5, 32.3, 34.4, 35.0, 44.9, 45.8, 56.7, 57.1, 58.5, 72.6, 81.2, 81.5, 81.6, 108.5, 108.7, 126.5, 126.9, 132.7, 133.8, 134.9, 135.8, 141.3, 145.1, 156.1, 168.3, 180.5, 183.9. ESI-TOF-MS *m/z* 645.3823, calcd for $C_{34}H_{53}N_4O_8$ ($[M+H]^+$) 645.3858.

5.1.17. 17-[3-(Diethylamino)propyl]amino-17-demethoxygeldanamycin (33). ^1H NMR: δ 0.96 (d, 3, $J = 6.4$ Hz), 0.99 (d, 3, $J = 6.8$ Hz), 1.06 (t, 6, $J = 7.2$ Hz), 1.71–1.80 (m, 6), 2.01 (s, 3), 2.44 (dd, 1, $J = 10.8$, 13.6 Hz), 2.58–2.75 (m, 8), 3.25 (s, 3), 3.35 (s, 3), 3.43–3.45 (m, 1), 3.55–3.60 (m, 2), 3.71–3.75 (m, 1), 4.30 (d, 1, $J = 10.0$ Hz), 4.48 (s, br, 1), 4.88 (s, br, 2), 5.16 (s, 1), 5.84 (t, 1, $J = 10.4$ Hz), 5.90 (d, 1, $J = 9.6$ Hz), 6.57 (t, 1, $J = 11.6$ Hz), 6.94 (t, 1, $J = 11.6$ Hz), 7.22 (s, 1), 7.77 (s, br, 1), 9.20 (s, 1). ^{13}C NMR: δ 11.1, 12.3, 12.6, 12.7, 22.8, 26.1, 28.4, 32.3, 34.5, 35.1, 46.1, 46.8, 51.3, 56.7, 57.1, 72.6, 81.3, 81.6, 81.7, 108.3, 108.8, 126.6, 126.8, 132.7, 133.9, 135.6, 141.3, 145.9, 156.1, 168.4, 180.0, 184.2. ESI-TOF-MS *m/z* 659.4010, calcd for $C_{35}H_{55}N_4O_8$ ($[M+H]^+$) 659.4014.

5.1.18. 17-[3-(1-Morpholinyl)propyl]amino-17-demethoxygeldanamycin (34). ^1H NMR: δ 0.97 (d, 3, $J = 6.4$ Hz), 1.01 (d, 3, $J = 7.2$ Hz), 1.74–1.86 (m, 3), 1.79 (s, 3), 2.02 (s, 3), 2.42–2.50 (m, 7), 2.68 (d, 1, $J = 14.4$ Hz), 2.71–2.76 (m, 1), 3.26 (s, 3), 3.36 (s, 3), 3.43–3.47 (m, 1), 3.53–3.60 (m, 2), 2.71–2.76 (m, 1), 3.80 (t, 6, $J = 4.8$ Hz), 4.31 (d, 1, $J = 10.0$ Hz), 4.41 (br s, 1), 4.80 (br s, 2), 5.18 (s, 1), 5.85 (t, 1, $J = 10.8$ Hz), 5.91 (d, 1, $J = 9.6$ Hz), 6.58 (t, 1, $J = 11.6$ Hz), 6.95 (d, 1, $J = 11.6$ Hz), 7.25 (s, 1), 7.49–7.51 (m, 1), 9.19 (s, 1). ^{13}C NMR: δ 12.3, 12.6, 12.8, 22.9, 25.2, 28.5, 32.3, 34.5, 35.2, 46.0, 53.9, 56.7, 57.1, 57.3, 66.7, 72.6, 81.2, 81.6, 81.7, 108.2, 108.8, 126.6, 126.8, 132.7, 133.8, 135.0, 135.7, 141.4, 145.7, 156.0, 168.4, 180.4, 184.0. ESI-TOF-MS *m/z* 673.3760, calcd for $C_{35}H_{53}N_4O_9$ ($[M+H]^+$) 673.3807.

5.1.19. 17-[2-(1-Methylimidazol-4-yl)ethyl]amino-17-demethoxygeldanamycin (36). ^1H NMR: δ 0.94 (d, 3, $J = 6.8$ Hz), 0.97 (d, 3, $J = 7.2$ Hz), 1.72–1.75 (m, 3), 1.77 (s, 3), 2.02 (s, 3), 2.44 (dd, 1, $J = 10.8$, 13.6 Hz), 2.64 (d, 1, $J = 13.6$ Hz), 2.71–2.75 (m, 1), 2.87 (t, 1, $J = 6.4$ Hz), 3.24 (s, 3), 3.34 (s, 3), 3.42 (m, 1), 3.55–3.60 (m, 1), 3.63 (s, 3), 3.74 (m, 1), 3.86 (m, 1), 4.28 (d, 1, $J = 9.6$ Hz), 4.34–4.4 0 (m, 1), 4.97 (br s, 2), 5.16 (s, 1), 5.83 (t, 1, $J = 10.4$ Hz), 5.88 (d, 1, $J = 9.6$ Hz), 6.56 (t, 1, $J = 11.6$ Hz), 6.70 (s, 1), 6.80 (m, 1), 6.93 (d, 1, $J = 11.6$ Hz), 7.20 (s, 1), 7.37 (s, 1), 9.17 (s, 1). ^{13}C NMR: δ 12.3, 12.6, 12.7, 22.9, 28.1, 28.5, 32.3, 33.3, 34.5, 35.0, 45.6, 56.7, 57.1, 72.6, 81.3, 81.5, 81.6, 108.6, 108.7, 117.5, 126.5, 126.9, 132.7, 133.8, 134.9, 135.7, 137.9, 138.6, 141.3, 145.4, 156.2, 168.4, 180.2, 184.1. ESI-TOF-MS *m/z* 654.3472, calcd for $C_{34}H_{48}N_5O_8$ ($[M+H]^+$) 654.3497.

5.1.20. 17-[2-(Carboxy)ethyl]amino-17-demethoxygeldanamycin (39). ^1H NMR (CD_3OD): δ (relative to CHD_2OD at 3.30 ppm) 0.96 (d, 3, $J = 6.8$ Hz), 1.00 (d, 3, $J = 6.4$ Hz), 1.61–1.80 (m, 3), 1.73 (s, 3), 1.99 (s, 3), 2.36 (dd, 1, $J = 13.6$, 9.2 Hz), 2.52 (m, 2), 2.72 (m, 2), 3.29 (s, 3), 3.34 (s, 3), 3.46 (m, 1), 3.59 (m, 1), 3.78 (m, 2), 4.53 (d, 1, $J = 8.0$ Hz), 5.19 (s, 1), 5.60 (d, 1, $J = 9.2$ Hz), 5.87 (t, 1, $J = 9.8$ Hz), 6.62 (t, 1, $J = 11.2$ Hz), 7.12 (d, 1, $J = 12.0$ Hz), 7.04 (s, 1). ^{13}C NMR (CD_3OD): δ (relative to CD_3OD at 49.0 ppm) 12.4, 13.5, 14.2, 22.6, 31.3, 33.7, 34.4, 35.6, 37.9, 56.8, 57.5, 74.3, 82.0 (2C), 82.9, 109.2, 109.7, 127.1, 129.6, 132.7, 134.4, 135.4, 137.9, 142.8, 146.8, 159.1, 170.7, 178.8, 180.9, 185.8. ESI-TOF-MS *m/z* 640.2868, calcd for $C_{31}H_{43}N_3O_{10}Na$ ($[M+Na]^+$) 640.2841.

5.1.21. 17-[2-(*N*-Methylethylamino)ethyl]amino-17-demethoxygeldanamycin (42). ^1H NMR: δ 0.92 (d, 3, $J = 6.8$ Hz), 0.94 (d, 3, $J = 6.8$ Hz), 1.05 (t, 3, $J = 7.2$ Hz), 1.61–1.80 (m, 3), 1.74 (s, 3), 1.98 (s, 3), 2.18 (s, 3), 2.36 (m, 1), 2.45 (q, 2, $J = 7.2$ Hz), 2.55–2.80 (m, 4), 3.20 (s, 3), 3.31 (s, 3), 3.42 (m, 2), 3.52 (m, 1), 3.64 (m, 1), 4.26 (d, 1, $J = 10.0$ Hz), 4.54 (br s, 1), 5.22 (br s, 2), 5.12 (s, 1), 5.86 (d, 1, $J = 9.2$ Hz), 5.80 (t, 1, $J = 10.6$ Hz), 6.54 (t, 1, $J = 11.4$ Hz), 6.90 (d, 1, $J = 11.6$ Hz), 7.11 (br s, 1), 7.19 (s, 1), 9.16 (s, 1). ^{13}C NMR: δ 12.1, 12.2, 12.5, 12.6, 22.8, 28.3, 32.1, 34.3, 35.0, 40.5, 42.4, 51.0, 54.6, 56.6, 56.9, 72.4, 81.1, 81.4 (2C), 108.4, 108.7, 126.5, 126.7, 132.6, 133.6, 134.8, 135.6, 141.1, 145.4, 156.3, 168.3, 179.8, 184.0. ESI-TOF-MS *m/z* 631.3688, calcd for $C_{33}H_{51}N_4O_8$ ($[M+H]^+$) 631.3701.

5.1.22. 17-[2-(*N*-Methyl-2-fluoroethylamino)ethyl]amino-17-demethoxygeldanamycin (43). ^1H NMR: δ 0.91 (d, 3, $J = 7.2$ Hz), 0.93 (d, 3, $J = 7.2$ Hz), 1.61–1.80 (m, 3), 1.73

(s, 3), 1.97 (s, 3), 2.29 (s, 3), 2.34 (m, 1), 2.55–2.80 (m, 6), 3.19 (s, 3), 3.30 (s, 3), 3.40 (m, 1), 3.43 (m, 1), 3.52 (m, 1), 3.64 (m, 1), 4.25 (d, 1, J = 10.0 Hz), 4.47 (br s, 1), 4.53 (dd, 2, J = 47.6, 4.8 Hz), 5.25 (br s, 2), 5.11 (s, 1), 5.84 (d, 1, J = 9.6 Hz), 5.79 (t, 1, J = 10.4 Hz), 6.53 (t, 1, J = 11.2 Hz), 6.89 (d, 1, J = 11.6 Hz), 7.02 (br s, 1), 7.17 (s, 1), 9.13 (s, 1). ^{13}C NMR: δ 12.2, 12.4, 12.6, 22.7, 28.3, 32.1, 34.1, 34.9, 41.3, 42.3, 55.4, 56.5, 56.8, 56.8 (d, $^{2}J_{C-F}$ = 19.5 Hz), 72.3, 81.1, 81.3 (2C), 82.1 (d, $^{1}J_{C-F}$ = 168 Hz), 108.6, 108.6, 126.4, 126.7, 132.6, 133.5, 134.8, 135.5, 141.0, 145.3, 156.3, 168.2, 179.9, 183.9. ESI-TOF-MS m/z 649.3626, calcd for $C_{33}H_{50}FN_4O_8$ ($[M+H]^+$) 649.3607.

5.1.23. 17-[2-(*N*-Methyl-2,2-difluoroethylamino)-ethyl]-amino-17-demethoxygeldanamycin (44). ^{1}H NMR: δ 0.94 (d, 3, J = 6.0 Hz), 0.97 (d, 3, J = 7.2 Hz), 1.61–1.80 (m, 3), 1.77 (s, 3), 2.00 (s, 3), 2.38 (s, 3), 2.40 (m, 1), 2.61–2.80 (m, 4), 2.82 (dt, 2, J = 14.8, 4 Hz), 3.24 (s, 3), 3.34 (s, 3), 3.44 (m, 1), 3.47 (m, 1), 3.55 (m, 1), 3.68 (m, 1), 4.29 (d, 1, J = 9.6 Hz), 4.43 (br s, 1), 5.05 (br s, 2), 5.15 (s, 1), 5.88 (d, 1, J = 9.2 Hz), 5.83 (t, 1, J = 10.8 Hz), 5.89 (tt, 1, J = 55.8, 4.0 Hz), 6.56 (t, 1, J = 11.2 Hz), 6.93 (d, 1, J = 9.6 Hz), 6.94 (br s, 1), 7.23 (s, 1), 9.15 (s, 1). ^{13}C NMR: δ 12.3, 12.5, 12.7, 22.9, 28.4, 32.2, 34.3, 35.0, 42.1, 44.4, 56.0, 56.6, 57.0, 59.0 (t, $^{2}J_{C-F}$ = 24.5 Hz), 72.5, 81.2, 81.4, 81.5, 108.8 (2C), 115.6 (t, $^{1}J_{C-F}$ = 242 Hz), 126.5, 126.8, 132.7, 133.7, 134.9, 135.7, 141.1, 145.2, 156.2, 168.3, 180.3, 184.0. ESI-TOF-MS m/z 667.3511, calcd for $C_{33}H_{49}F2N_4O_8$ ($[M+H]^+$) 667.3513.

5.1.24. 17-[2-(Diethylamino)ethyl]amino-17-demethoxygeldanamycin (45). ^{1}H NMR: δ 0.94 (d, 6, J = 6.8 Hz), 1.00 (q, 6, J = 7.0 Hz), 1.61–1.80 (m, 3), 1.75 (s, 3), 1.98 (s, 3), 2.25–2.75 (m, 4), 2.50 (t, 4, J = 7.0 Hz), 3.21 (s, 3), 3.32 (s, 3), 3.40 (m, 1), 3.40 (m, 1), 3.53 (m, 1), 3.60 (m, 1), 4.26 (d, 1, J = 10.0 Hz), 4.58 (br s, 1), 5.21 (br s, 2), 5.13 (s, 1), 5.87 (d, 1, J = 8.8 Hz), 5.81 (t, 1, J = 10.0 Hz), 6.54 (t, 1, J = 11.4 Hz), 6.91 (d, 1, J = 11.2 Hz), 7.21 (br s, 1), 7.19 (s, 1), 9.17 (s, 1). ^{13}C NMR: δ 11.6 (2C), 12.2, 12.5, 12.6, 22.9, 28.3, 32.1, 34.3, 35.0, 42.6, 46.2 (2C), 50.7, 56.6, 56.9, 72.4, 81.1, 81.4 (2C), 108.3, 108.7, 126.5, 126.7, 132.6, 133.6, 134.8, 135.5, 141.1, 145.4, 156.3, 168.3, 179.7, 184.0. ESI-TOF-MS m/z 645.3882, calcd for $C_{34}H_{53}N_4O_8$ ($[M+H]^+$) 645.3858.

5.1.25. 17-[2-(Aziridinyl)ethyl]amino-17-demethoxygeldanamycin (46). ^{1}H NMR: δ 0.93 (d, 6, J = 6.8 Hz), 1.16 (m, 2), 1.61–1.80 (m, 3), 1.77 (m, 2), 1.73 (s, 3), 1.97 (s, 3), 2.31–2.50 (m, 3), 2.55–2.75 (m, 2), 3.20 (s, 3), 3.31 (s, 3), 3.39 (m, 1), 3.59 (m, 1), 3.54 (m, 1), 3.71 (m, 1), 4.25 (d, 1, J = 10.0 Hz), 4.40 (br s, 1), 5.23 (br s, 2), 5.11 (s, 1), 5.84 (d, 1, J = 8.8 Hz), 5.80 (t, 1, J = 10.8 Hz), 6.53 (t, 1, J = 11.4 Hz), 6.90 (d, 1, J = 11.2 Hz), 6.82 (br s, 1), 7.20 (s, 1), 9.13 (s, 1). ^{13}C NMR: δ 12.2, 12.4, 12.6, 22.7, 27.0, 27.2, 28.3, 32.1, 34.2, 34.9, 45.3, 56.5, 56.8, 59.5, 72.4, 81.1, 81.3 (2C), 108.4, 108.6, 126.4, 126.8, 132.7, 133.5, 134.8, 135.6, 141.0, 145.0, 156.3, 168.2, 180.2, 183.7. ESI-TOF-MS m/z 615.3362, calcd for $C_{32}H_{47}N_4O_8$ ($[M+H]^+$) 615.3388.

5.1.26. 17-[2-(Azetidinyl)ethyl]amino-17-demethoxygeldanamycin (47). ^{1}H NMR: δ 0.95 (d, 3, J = 6.8 Hz), 0.99 (d, 3, J = 6.8 Hz), 1.61–1.80 (m, 3), 1.79 (s, 3), 2.02 (s, 3), 2.11 (m, 2), 2.41 (dd, 1, J = 11.2, 13.6 Hz), 2.61–2.80 (m, 2), 2.66 (t, 2, J = 6.0 Hz), 3.23 (t, 4, J = 7.0 Hz), 3.25 (s, 3), 3.35 (s, 3), 3.43 (m, 1), 3.38 (m, 1), 3.56 (m, 1), 3.56 (m, 1), 4.30 (d, 1, J = 10.0 Hz), 4.50 (br s, 1), 4.90 (br s, 2), 5.17 (s, 1), 5.91 (d, 1, J = 9.2 Hz), 5.84 (t, 1, J = 10.4 Hz), 6.58 (t, 1, J = 11.4 Hz), 6.94 (d, 1, J = 11.6 Hz), 6.89 (br s, 1), 7.25 (s, 1), 9.18 (s, 1). ^{13}C NMR: δ 12.2, 12.4, 12.6, 17.4, 22.7, 28.3, 32.1, 34.1, 34.9, 43.0, 54.8 (2C), 56.5, 56.8, 57.3, 72.4, 81.1, 81.3 (2C), 108.4, 108.6, 126.4, 126.7, 132.6, 133.5, 134.8, 135.6, 141.0, 145.2, 156.3, 168.2, 179.9, 183.8. ESI-TOF-MS m/z 629.3558, calcd for $C_{33}H_{49}N_4O_8$ ($[M+H]^+$) 629.3545.

5.1.27. 17-[2-(Pyrrolidin-1-yl)ethyl]amino-17-demethoxygeldanamycin (48). ^{1}H NMR: δ 0.92 (d, 3, J = 6.4 Hz), 0.94 (d, 3, J = 6.8 Hz), 1.61–1.80 (m, 7), 1.74 (s, 3), 1.97 (s, 3), 2.36 (dd, 1, J = 11.6, 13.6 Hz), 2.49 (m, 4), 2.62 (d, 1, J = 13.6 Hz), 2.71 (m, 3), 3.14 (s, 3), 3.31 (s, 3), 3.42 (m, 1), 3.37 (m, 1), 3.53 (m, 1), 3.66 (m, 1), 4.25 (d, 1, J = 10.0 Hz), 4.54 (br s, 1), 5.24 (br s, 2), 5.12 (s, 1), 5.86 (d, 1, J = 9.2 Hz), 5.80 (t, 1, J = 10.4 Hz), 6.54 (t, 1, J = 11.4 Hz), 6.90 (d, 1, J = 11.6 Hz), 7.04 (br s, 1), 7.19 (s, 1), 9.15 (s, 1). ^{13}C NMR: δ 12.2, 12.5, 12.6, 22.8, 23.4 (2C), 28.3, 32.1, 34.2, 34.9, 43.9, 53.3 (2C), 53.8, 56.6, 56.9, 72.4, 81.1, 81.4 (2C), 108.4, 108.6, 126.5, 126.7, 132.6, 133.6, 134.8, 135.6, 141.0, 145.4, 156.3, 168.3, 179.8, 184.0. ESI-TOF-MS m/z 643.3721, calcd for $C_{34}H_{51}N_4O_8$ ($[M+H]^+$) 643.3701.

5.1.28. 17-[2-(Piperazin-1-yl)ethyl]amino-17-demethoxygeldanamycin (49). ^{1}H NMR: δ 0.96 (d, 3, J = 6.4 Hz), 0.99 (d, 3, J = 7.2 Hz), 1.68–1.78 (m, 3), 1.79 (s, 3), 2.02 (s, 3), 2.39 (m, 1), 2.46 (m, 4), 2.58 (m, 2), 2.61–2.75 (m, 2), 2.93 (t, 4, J = 4.6 Hz), 3.25 (s, 3), 3.35 (s, 3), 3.44–3.52 (m, 2), 3.57 (m, 1), 3.68 (m, 1), 4.30 (d, 1, J = 10.0 Hz), 4.94 (br s, 2), 5.17 (s, 1), 5.85 (t, 1, J = 10.4 Hz), 5.91 (d, 1, J = 9.6 Hz), 6.58 (t, 1, J = 11.4 Hz), 6.94 (d, 1, J = 11.6 Hz), 7.15 (br s, 1), 7.25 (s, 1), 9.18 (s, 1). ^{13}C NMR: δ 12.3, 12.6, 12.7, 23.1, 28.4, 32.3, 34.4, 35.1, 41.6, 45.8, 53.4, 56.2, 56.7, 57.1, 72.5, 81.2, 81.5, 81.7, 108.7, 108.9, 126.6, 126.8, 132.7, 133.8, 135.0, 135.7, 141.2, 145.5, 156.1, 168.4, 180.1, 184.1. ESI TOF MS m/z 658.3820, calcd for $C_{34}H_{52}N_5O_8$ ($[M+H]^+$) 658.3810.

5.1.29. 17-[2-(Morpholin-4-yl)ethyl]amino-17-demethoxygeldanamycin (50). ^{1}H NMR: δ 0.95 (d, 3, J = 6.0 Hz), 0.98 (d, 3, J = 6.8 Hz), 1.71–1.80 (m, 3), 1.77 (s, 3), 2.01 (s, 3), 2.38 (m, 1), 2.46 (m, 4), 2.64 (m, 2), 2.61–2.75 (m, 2), 3.24 (s, 3), 3.34 (s, 3), 3.44–3.52 (m, 2), 3.56 (m, 1), 3.68 (m, 1), 3.73 (m, 4), 4.29 (d, 1, J = 9.6 Hz), 4.47 (br s, 1), 4.99 (br s, 2), 5.15 (s, 1), 5.84 (t, 1, J = 10.4 Hz), 5.88 (d, 1, J = 9.6 Hz), 6.57 (t, 1, J = 11.4 Hz), 6.93 (d, 1, J = 11.2 Hz), 7.10 (br s, 1), 7.24 (s, 1), 9.17 (s, 1). ^{13}C NMR: δ 12.3, 12.6, 12.7, 23.0, 28.4, 32.2, 34.4, 35.0, 41.4, 56.1, 56.7, 56.7, 57.0, 66.8, 72.5, 81.1, 81.4, 81.6, 108.8, 108.8, 126.5, 126.8, 132.7, 133.7, 134.9, 135.7, 141.1, 145.3, 156.2, 168.4,

180.2, 184.0. ESI-TOF-MS *m/z* 659.3662, calcd for $C_{34}H_{51}N_4O_9$ ($[M+H]^+$) 659.3651.

5.1.30. 17-[2-(Acetylamino)ethyl]amino-17-demethoxygeldanamycin (53). ^{1}H NMR: δ 0.93 (d, 3, J = 7.2 Hz), 0.95 (d, 3, J = 7.2 Hz), 1.61–1.80 (m, 3), 1.76 (s, 3), 1.99 (s, 3), 2.00 (s, 3), 2.35 (dd, 1, J = 11.2, 13.6 Hz), 2.39 (d, 1, J = 14.0 Hz), 2.71 (m, 1), 3.23 (s, 3), 3.32 (s, 3), 3.40 (m, 1), 3.50 (m, 2), 3.54 (m, 1), 3.69 (m, 2), 4.27 (d, 1, J = 10.0 Hz), 4.33 (br s, 1), 5,14 (br s, 2), 5.11 (s, 1), 5.84 (d, 1, J = 10.0 Hz), 5.83 (t, 1, J = 10.8 Hz), 6.55 (t, 1, J = 11.6 Hz), 6.91 (d, 1, J = 11.6 Hz), 6.76 (br s, 1), 7.15 (s, 1), 9.10 (s, 1). ^{13}C NMR: δ 12.2, 12.5, 12.7, 22.7, 23.0, 28.4, 32.1, 34.1, 34.9, 39.3, 46.5, 56.6, 57.0, 72.4, 81.2, 81.3, 81.7, 108.8, 109.5, 126.5, 126.9, 132.9, 133.6, 134.8, 135.8, 140.9, 145.6, 156.3, 168.2, 171.7, 180.2, 184.2. ESI-TOF-MS *m/z* 629.3175, calcd for $C_{32}H_{45}N_4O_9$ ($[M-H]^-$) 629.3181.

5.1.31. 17-{2-[(Dimethylamino)methyl]aziridin-1-yl}-17-demethoxygeldanamycin, the less polar diastereomer from racemic 2-[(dimethylamino)methyl]aziridine (58). ^{1}H NMR: δ 0.96 (d, 3, J = 6.8 Hz), 0.99 (d, 3, J = 5.6 Hz), 1.75–1.80 (m, 3), 1.78 (s, 3), 2.01 (s, 3), 2.01–2.10 (m, 1), 2.32 (s, 6), 2.32–2.80 (m, 5), 2.75 (m, 1), 3.20 (dd, 1, J = 10.8, 2.8 Hz), 3.29 (s, 3), 3.34 (s, 3), 3.39 (m, 1), 3.50 (m, 1), 4.31 (d, 1, J = 9.2 Hz), 4.90 (br s, 2), 5.16 (s, 1), 5.80 (d, 1, J = 9.2 Hz), 5.86 (t, 1, J = 10.2 Hz), 6.55 (t, 1, J = 11.4 Hz), 6.91 (d, 1, J = 11.6 Hz), 7.27 (s, 1), 8.77 (s, 1). ^{13}C NMR: δ 12.4, 12.5, 12.8, 23.3, 28.7, 32.2, 33.5, 34.6, 35.8, 38.9, 46.0 (2C), 56.7, 57.3, 61.2, 72.8, 81.1, 81.3, 81.7, 111.7, 125.3, 126.3, 127.0, 133.1, 133.1, 134.9, 136.3, 138.8, 152.8, 156.0, 168.3, 183.2, 183.9. ESI-TOF-MS *m/z* 629.3526, calcd for $C_{33}H_{49}N_4O_8$ ($[M+H]^+$) 629.3545.

5.1.32. 17-{2-[(Dimethylamino)methyl]aziridin-1-yl}-17-demethoxygeldanamycin, the more polar diastereomer from racemic 2-[(dimethylamino)methyl]aziridine (59). ^{1}H NMR: δ 0.93 (d, 6, J = 5.6 Hz), 1.55–1.80 (m, 3), 1.72 (s, 3), 1.95 (s, 3), 2.01–2.10 (m, 1), 2.27 (s, 6), 2.15–2.60 (m, 5), 2.68 (m, 1), 3.25 (m, 1), 3.21 (s, 3), 3.30 (s, 3), 3.36 (m, 1), 3.48 (m, 1), 4.26 (d, 1, J = 9.6 Hz), 5.24 (br s, 2), 5.13 (s, 1), 5.76 (d, 1, J = 8.4 Hz), 5.80 (t, 1, J = 10.2 Hz), 6.51 (t, 1, J = 11.2 Hz), 6.87 (d, 1, J = 11.2 Hz), 7.19 (s, 1), 8.73 (s, 1). ^{13}C NMR: δ 12.4 (2C), 12.7, 23.1, 28.9, 32.2, 33.5, 35.0, 35.4, 39.2, 45.9 (2C), 56.5, 57.0, 61.4, 72.5, 80.9, 81.0, 81.2, 111.5, 125.0, 126.2, 126.9, 133.0 (2C), 134.7, 136.0, 138.6, 152.7, 156.2, 168.2, 183.0, 183.8. ESI-TOF-MS *m/z* 629.3560, calcd for $C_{33}H_{49}N_4O_8$ ($[M+H]^+$) 629.3545.

5.1.33. 17-(3-Dimethylaminoazetidin-1-yl)-17-demethoxygeldanamycin (61).[36] ^{1}H NMR: δ 0.94 (m, 6H), 1.64 (m, 1H), 1.75 (m, 5H), 1.98 (s, 3H), 2.16 (s, 6H), 2.22 (m, 1H), 2.58 (m, 1H), 2.69 (m, 1H), 3.08 (m, 1H), 3.21 (s, 3H), 3.32 (s, 3H), 3.40 (m, 1H), 3.53 (m, 1H), 4.26 (d, 1H, J = 10.0 Hz), 4.38 (m, 2H), 4.59 (m, 2H), 5.13 (s, 1H), 5.81 (t, 1H, J = 10.4 Hz), 5.87 (d, 1H, J = 9.2 Hz), 6.54 (t, 1H, J = 11.20 Hz), 6.91 (d, 1H, J = 11.6 Hz), 7.08 (s, 1H), 9.12 (s, 1H). ^{13}C NMR: δ 12.2, 12.5, 12.7, 22.96, 28.1, 32.2, 34.0, 35.0, 41.8, 56.6, 56.9, 57.2, 62.5, 72.4, 81.2, 81.5, 81.5, 109.6, 109.6, 126.5, 126.7, 132.6, 133.7, 134.9, 135.5, 140.3, 145.5, 156.3, 168.4, 178.7, 185.7. ESI-TOF-MS *m/z* 629.3557, calcd for $C_{33}H_{49}N_4O_8$ ($[M+H]^+$) 629.3545.

5.1.34. Solubility measurements. Solubility limits were estimated as the concentrations obtained in saturated solutions. GA analogs (~3 mg) were stirred at room temperature in the dark for 1 or 4 days in 0.2 mL of 50 mM sodium phosphate, pH 7.0. The resulting solutions were clarified two times by centrifugation, and concentrations were measured spectrophotometrically following 100-fold dilution into methanol using ε_{332} = 22,200 $M^{-1}cm^{-1}$.

5.2. Biological evaluation

5.2.1. Cells. The human breast cancer cell line SKBr3 was obtained from the American Type Culture Collection (Manassas, VA) and maintained in McCoy's 5A modified medium (Invitrogen; Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) (Hyclone; Logan, UT) and 2 mM glutamine, in humidified air with 5% CO_2 at 37 °C.

5.2.2. Cell growth inhibition assay. Cells were seeded in duplicate in 96-well black tissue culture microtiter plates at ~4000 cells per well and allowed to attach overnight. Serial 10-fold dilutions of compounds were added, and the cells were incubated for 72 h. Cell viability was determined using the CellTiter-Glo™Luminescent Cell Viability Assay (Promega; Madison, WI). IC_{50} is defined as the concentration of drug required for inhibiting cell growth by 50%.

5.2.3. Scintillation Proximity Assay for Hsp90 binding. Truncated[28] and full-length forms of human Hsp90 were expressed in *E. coli* with *N*-terminal polyHis and biotinylation recognition sequence (BRS) tags to facilitate purification and SPA assay, respectively. The tagged full-length Hsp90 sequence consisted of the natural Hsp90α sequence[37] preceded by the sequence MSH_{10}SLTDIFEAQKIEWHHMA where the BRS is underlined. The 3′-end of the gene contained a *Bam*HI site, adding a single proline residue to the C-terminus. The tagged full-length protein was cloned into pET21d as described for the tagged *N*-domain of Hsp90,[28] and co-expressed with biotin ligase encoded by pBIRAcm (Avidity, Denver, CO) in *Escherichia coli* BL21DE(3).

Biotinylated Hsp90 was added to a 1 mg/mL suspension of streptavidin coated YiSi beads (Pharmacia RPNQ0012; 219 pmol streptavidin/mg) in binding buffer (10 mM Tris–HCl, 5 mM $MgCl_2$, pH 7.0) to obtain a final concentration of 225 nM. [*allyl*-^{3}H]-17-AAG (2000 cpm/pmol) was then added to a final concentration of 2 μM, and 50 μL aliquots of the resulting suspension were mixed with 50 μL aliquots of 0.1–50 μM test compounds in binding buffer. Reaction mixtures were incubated for 2~4 h at room temperature in 96-well assay plates, then signals for each reaction were measured using a Wallac Microbeta scintillation counter. The resulting data was fit to a competitive binding equation.[38]

Acknowledgements

We thank John R. Carney and Nina Viswanathan for the analytical chemistry support, Wei Ma and Li Feng for their assistance in cytotoxicity assays, Kwok Yu, Ziyang Zhong, Andreas Schirmer for their assistance in the Hsp90 binding assays, Courtney Starks for providing geldanamycin, and Thomas Mueller for helpful discussions.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2004.07.053. Information on synthesis of amines unavailable commercially and analytical data on additional compounds.

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