Synthesis and anticancer activity of geldanamycin derivatives derived from biosynthetically generated metabolites

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A new series of geldanamycin derivatives were synthesized using a semi-synthetic approach involving genetically engineered biosynthetic intermediates. These analogues were then evaluated for anti-proliferation activity in human cancer cell lines, SK-Br3 and SK-Ov3. Most of the synthesized compounds exhibited potent *in vitro* anti-proliferation activity toward both cell lines. Such compounds potently inhibited the expression of the Hsp90 client protein ErbB2.

Introduction

Heat shock protein 90 (Hsp90), a molecular chaperone, represents a promising target for future cancer chemotherapy, due to its importance in maintaining transformation, and increasing the survival and growth potentials of cancer cells.[1–5] Hsp90 inhibition results in the blocking of Hsp90-mediated conformational maturation–refolding of Hsp90 client proteins, which ultimately leads to proteasomal degradation of the clients.[6] The clients come from a variety of signaling pathways and include mutated p53, mitogen-activated protein kinase kinases (MEK1 and 2), Raf, Akt, Bcr-Abl, hypoxia-inducible factor 1α (HIF-1α) and ErbB2.[7,8] In particular, ErbB2 (also known as Her2) is over-expressed in many human tumors, including approximately >25% of human breast cancers.[9,10]

Geldanamycin (**1**, Fig. 1), a naturally occurring antitumor antibiotic, inhibits Hsp90 by competing with ATP for a highly conserved nucleotide binding site located near the N-terminus of the protein, and the resulting ATP-dependent chaperone activities are thus inhibited.[11] Geldanamycin has exhibited potent anti-proliferative activity in various cancer cell lines[12] and has been shown to inhibit tumor growth in mouse xenograft models.[13] However, no clinical evaluation of geldanamycin has been undertaken because of its severe toxicity and poor water solubility.[14] Thus far, a number of geldanamycin derivatives have been prepared, among which 17-allylamino-17-demethoxygeldanamycin (17-AAG) **2**, and 17-[2-(dimethylamino)ethyl]amino-17-demethoxygeldanamycin (17-DMAG) **3** are currently in various stages of clinical trials for the treatment of cancer.[15–19]

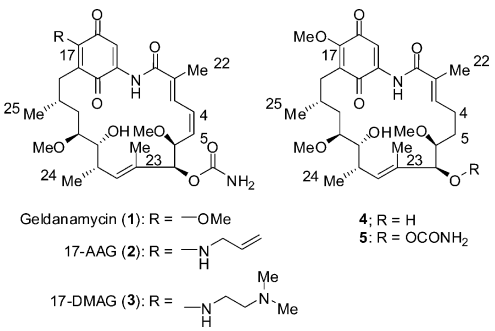

Fig. 1 Geldanamycin and related analogues.

The preparation of geldanamycin and its derivatives with the benzoquinone–ansamycin moiety have been achieved by either synthetic[20–28] or biosynthetic routes.[29,30] In particular, a biosynthetic route, including alteration of the gene for the biosynthesis of the geldanamycin macrocyclic ring, provided some interesting analogues that are difficult to prepare synthetically.[29] Moreover, biosynthetic analogues, such as 4,5-dihydro-7-*O*-descarbamoyl-7-hydroxygeldanamycin **4** and 4,5-dihydrogeldanamycin **5**, obtained from a genetically engineered mutant containing a selective inactivation of a post-polyketide synthase modification step,[31,32] may be utilized as unique templates for access to novel geldanamycin derivatives.

Herein, we report a semi-synthetic preparation of a new series of geldanamycin derivatives from geldanamycin (**1**) and the biosynthetically generated metabolites, **4** and **5**, and their anticancer activities.

Results and discussion

Chemistry

Recently, we have reported that **4** is the main product of a culture using a carbamoyltransferase gene-inactivated strain of *Streptomyces hygroscopicus* subsp. *duamyceticus* JCM4427 in the biosynthesis of geldanamycin.[31] More recently, we have found that **5** is the final biosynthetic intermediate of the geldanamycin biosynthetic pathway, and can be readily purified in a high-sucrose containing culture broth (Fig. 2).[33] With these biosynthetics **1**, **4**, and **5** in hand, a variety of geldanamycin derivatives were synthesized as outlined in Schemes 1–3.

As shown in Scheme 1, reaction of compound **4** with trichloroacetyl isocyanate gave bis-carbamoyl derivative **6**, which was further treated with various amines to produce the 17-substituted

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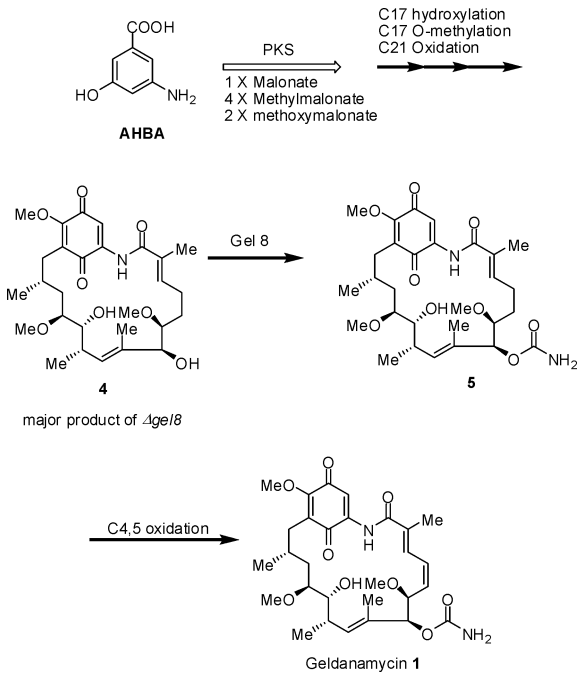
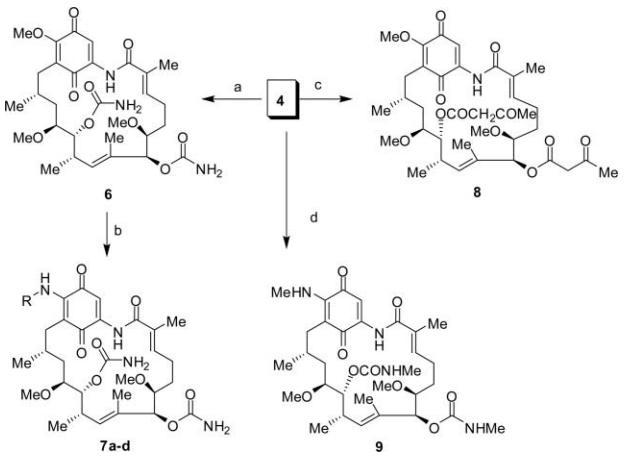
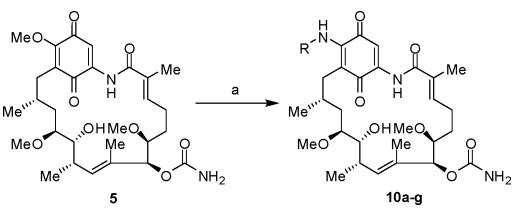


Fig. 2 Proposed geldanamycin biosynthetic pathway, following polyketide synthase (PKS) processing and modification by PKS tailoring enzymes. Compound **4** was converted to 4,5-dihydrogeldanamycin (**5**) by a carbamoyltransferase (Gel8).

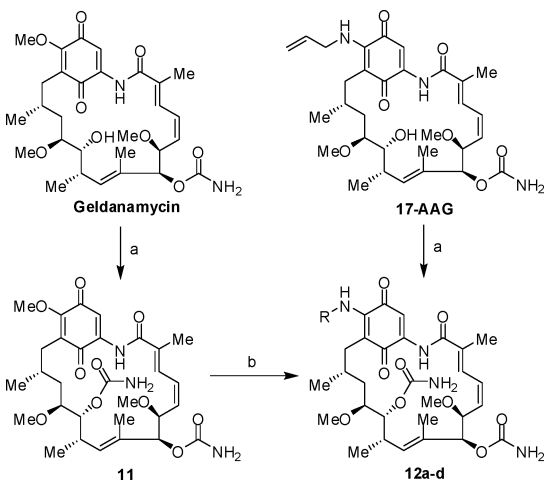


Scheme 1 *Reagents and conditions*: (a) trichloroacetyl isocyanate, CH_2Cl_2, 0 °C–RT, Al_2O_3; (b) amine, DCE, RT; (c) diketene, DMAP, Et_3N, THF, RT; (d) 1,1′-carbonyldiimidazole, CH_2Cl_2, RT, 12 h, then methylamine, THF, RT, 1 h.



Scheme 2 *Reagents and conditions*: (a) amine, DCE, RT.

amino analogues **7a–d**. A single step reaction of **4** with diketene afforded the bis-diketone derivative **8** in good yield. In addition,



Scheme 3 *Reagents and conditions*: (a) trichloroacetyl isocyanate, CH_2Cl_2, 0 °C–RT, Al_2O_3; (b) amine, DCE, RT.

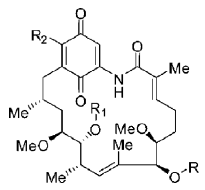
4 was reacted with 1,1′-carbonyldiimidazole for 12 hours and the resulting intermediate was further treated with methylamine to yield the bis-methylamino carbamoyl analogue **9**. Treatment of 4,5-dihydrogeldanamycin **5** with a series of aliphatic amines under standard conditions furnished 17-amino-17-demethoxy analogues of 4,5-dihydrogeldanamycin **10a–g** (Scheme 2). Furthermore, geldanamycin was reacted with trichloroacetyl isocyanate to obtain the corresponding 7,11-bis-carbamoyl derivative **11**, which on further treatment with various amines provided the respective amino analogues **12a–d**. Alternatively, **12a** was prepared in a single step by the carbamoylation of 17-AAG with trichloroacetyl isocyanate, as described in Scheme 3.

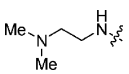
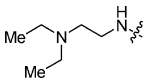
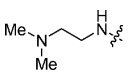
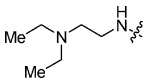
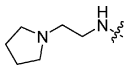
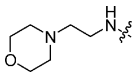
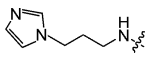
Biological evaluation

Inhibition of Hsp90 leads to the proteasomal degradation of a subset of signaling proteins that require Hsp90 chaperone activity for their conformational maturation.[34,35] Among these client proteins, ErbB2 is considered as a highly sensitive target of Hsp90 inhibitors. ErbB2 is a transmembrane tyrosine kinase whose surface over-expression is linked to tumorigenesis and poor prognosis in breast cancer patients. Breast cancers with high levels of ErbB2 expression are associated with aggressive disease and resistance to chemotherapy-induced apoptosis. Recently, it was reported that cells with ErbB2 over-expressed are 10- to 100-fold more sensitive to 17-AAG, **2**, than cancer cells with low expression levels of ErbB2,[9] and the concentrations of Hsp90 inhibitors that are needed to induce ErbB2 degradation match those required to impair cell proliferation.[10]

Initially, the newly prepared analogues were evaluated for their *in vitro* biological activity using tumor cell growth inhibition assays in human breast SK-Br3 and ovarian SK-Ov3 cancer cell lines, following the standard procedures.[20,21] The anti-proliferative activities of these analogues, along with those of the biosynthetic **4** and **5**, are summarized in Tables 1 and 2. **1–3** were used as reference standards. Among the series of 4,5-dihydro analogues, compound **4** with a 7-hydroxyl group was found to be inactive and the corresponding carbamate **5** exhibited activity, which is in accordance with the findings from the co-crystal structure of Hsp90·inhibitor.[36,37] That is, the 7-carbamate group of

Table 1 *In vitro* tumor cell growth inhibition data for 4,5-dihydrogeldanamycin **5** and its analogues **4** and **6–10**



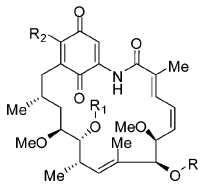
Compd	R	R_1	R_2	$IC_{50}/\mu M$ SK-Br3	$IC_{50}/\mu M$ SK-Ov3
4	H	H	OMe	>10	>10
5	$CONH_2$	H	OMe	1.4	>10
6	$CONH_2$	$CONH_2$	OMe	3.07	7.90
7a	$CONH_2$	$CONH_2$		>10	>10
7b	$CONH_2$	$CONH_2$		>10	10.52
7c	$CONH_2$	$CONH_2$		0.32	5.09
7d	$CONH_2$	$CONH_2$		0.01	1.14
8	$COCH_2COMe$	$COCH_2COMe$	OMe	>10	11.94
9	CONHMe	CONHMe	NHMe	>10	>10
10a	$CONH_2$	H		>10	>10
10b	$CONH_2$	H		1.02	10.22
10c	$CONH_2$	H		0.03	1.54
10d	$CONH_2$	H		0.02	1.66
10e	$CONH_2$	H		0.2	1.32
10f	$CONH_2$	H		0.83	4.12
10g	$CONH_2$	H		0.69	5.09

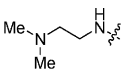
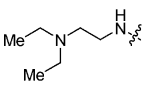
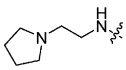
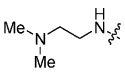
geldanamycin or its derivatives is found to be essential for biological activity, since this functionality makes important hydrogen-bonding networks at the active site of the Hsp90 protein. 7,11-Bis-carbamates **7a–d** with 17-aminoalkyl substitutions showed various activities.

The *in vitro* tumor cell growth inhibition data profile for 4,5-dihydrogeldanamycin **5** and its analogues **4** and **6–10** showed that compounds **7c** and **7d**, with diaminoalkyl functionality introduced at the 17-position, exhibited improved potency over compound **6**. Further structural modifications at the 7- and 11-positions with bis-ester (**8**) and bis-*N*-methylcarbamate (**9**) did not show any improvement in anti-proliferative activity, presumably because of their bulkiness. Other 4,5-dihydrogeldanamycin derivatives **10a–g** with a 7-carbamate were also found to have notable IC_{50} values, which displayed better activities than the corresponding 7,11-bis-carbamates, as exemplified by **7c** *vs.* **10c** (Table 1).

As shown in Table 2, geldanamycin derivatives **12a–d**, which have an additional carbamate group at the 11-position in comparison with the reference compounds **1–3**, also showed anti-proliferative activity in a similar range, with **12b** being the most potent in this series. Further with the difference at the 4–5 position, **12a** and **12b** were more potent than the corresponding 4,5-saturated compounds **7b** and **7c**, respectively. An exception to this trend was **12c** *vs.* **7d** (Table 2).

Table 2 *In vitro* tumor cell growth inhibition data for geldanamycin, 17-AAG, 17-DMAG and their analogues **12a–d**



Compd	R	R_1	R_2	$IC_{50}/\mu M$ SK-Br3	SK-Ov3
12a	$CONH_2$	$CONH_2$		0.05	6.97
12b	$CONH_2$	$CONH_2$		0.02	0.67
12c	$CONH_2$	$CONH_2$		>10	2.95
12d	$CONH_2$	$CONH_2$		0.51	2.07
17-AAG (**2**)	$CONH_2$	H		0.02	5.16
17-DMAG (**3**)	$CONH_2$	H		0.01	0.04
Geldanamycin (**1**)	$CONH_2$	H	OMe	0.89	7.66

In addition to anti-proliferation activity studies, we further examined whether ErbB2, a well-documented client of Hsp90, is degraded by representative analogues. Accordingly, we determined the TGI (total growth inhibition) concentrations for three selected compounds; **7d**, **12b** and **12d**, in the SK-Ov3 cell line, and then performed ErbB2 degradation studies to test whether ErbB2 levels are changed by these compounds at their TGI concentrations. These three compounds were found to have higher TGI concentrations than **3** (7.5 μM). Among the three, the most active compound was **12d** (9.7 μM), whereas **7d** and **12b** were less active with TGI concentrations of 14 μM and 17 μM, respectively. As shown in Fig. 3, western blot analysis indicated that ErbB2 was degraded by the inhibitors in a time-dependent manner. Of note, it took 4 hours for **12b** to markedly degrade ErbB2, while other inhibitors, including **3**, needed 8 hours. Therefore, we concluded that **12b** appeared to have a rapid onset anti-proliferative effect *via* Hsp90 inhibition in SK-Ov3 cells. Compound **12d** also needed 8 hours to completely deplete ErbB2 protein at the concentration of 9.7 μM. Furthermore, these findings further confirmed the potent anticancer properties *via* the inhibition of Hsp90 activity.

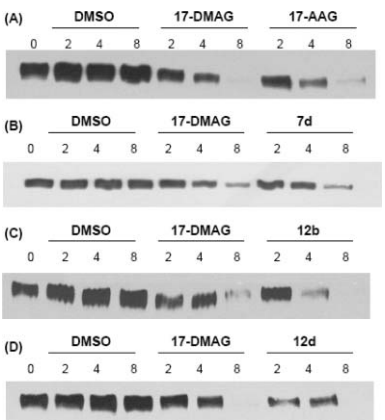


Fig. 3 Western blot analyses of analogues **7d**, **12b**, **12d**, 17-AAG (**2**) and 17-DMAG (**3**) in human ovarian cancer cell line SK-Ov3. Cells were treated with each compound at its TGI concentration (**2**, 1.5 μM; **3**, 7.5 μM; **7d**, 14 μM; **12b**, 17 μM; **12d**, 9.7 μM, respectively) for 0, 2, 4, and 8 h. The ErbB2 level was probed by western blot analysis of 30 μg of drug treated SK-Ov3 cell lysate. ErbB2 was remarkably reduced after 4 h of treatment with **12b** (C), and disappeared after 8 h of treatment with **12b** and **12d** (C and D, respectively).

Experimental

Synthetic procedures

All of the commercial chemicals and solvents were of reagent grade and were used without further purification. All reactions were carried out under an atmosphere of dried argon, in flame-dried glassware. Proton nuclear magnetic resonance (^{1}H NMR) spectra were determined on a Varian (300 MHz) spectrometer. Chemical shifts are provided in parts per million (ppm) downfield from tetramethylsilane (internal standard) with coupling constants in hertz (Hz). Multiplicity is indicated by the following abbreviations: singlet (s), doublet (d), doublet of doublets (dd), triplet (t),

pseudo triplet (ps-t), quartet (q), multiplet (m), broad (br). Mass spectra were recorded on a Finnigan ESI mass spectrometer and HRMS (EI-MS) was obtained on a JMS-700 (Jeol, Japan) mass spectrometer. Products from all reactions were purified to a minimum purity of 96% as determined by HPLC, either by flash column chromatography using silica gel 60 (230–400 mesh Kieselgel 60) or by preparative thin layer chromatography using glass-backed silica gel plates (1 mm thickness) unless otherwise indicated. Additionally, thin-layer chromatography on 0.25 mm silica plates (E. Merck, silica gel 60 F_{254}) was used to monitor reactions. The chromatograms were visualized using ultraviolet illumination, exposure to iodine vapors, dipping in PMA or Hanessian's solution. The purity of the products was checked by reversed phase high-pressure liquid chromatography (RP-HPLC), which was performed either on a Dionex Corp. HPLC system or on a Waters Corp. HPLC system equipped with a UV detector set at 254 nm. The mobile phases used were A: H_2O containing 0.05% TFA, and B: CH_3CN. The HPLC employed a YMC Hydrosphere C18 (HS-302) column (5 μ particle size, 12 nM pore size), 4.6 mm dia. × 150 mm with a flow rate of 1.0 mL min^{-1}. Compound purity was assessed in two different systems, using one of the following methods, method A: gradient 20% B to 100% B in 30 min (Waters Corp. HPLC system); method B: gradient 25% B to 100% B in 30 min (Dionex Corp. HPLC system).

11-Carbamate-4,5-dihydrogeldanamycin 6

To a solution of **4** (44.5 mg, 0.086 mmol) in CH_2Cl_2 (2.0 mL) was added trichloroacetyl isocyanate (0.010 mL, 0.086 mmol) at 0 °C and the reaction was stirred at room temperature for 1.5 h. The reaction mixture was diluted with CH_2Cl_2 and Al_2O_3 was added. The mixture was stirred for 2 h, filtered and concentrated *in vacuo*. Purification by preparative TLC (*n*-hexane–EtOAc–MeOH = 6 : 3 : 1) gave **6** as a yellow solid (35.1 mg, 68% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.64 (1H, s, N*H*), 7.05 (1H, s, 19-*H*), 6.52 (1H, t, J = 7.2 Hz, 3-*H*), 5.20 (1H, d, J = 10.5 Hz, 9-*H*), 4.90 (1H, d, J = 5.4 Hz, 7-*H*), 4.78 (2H, brs, NH_2), 4.51 (1H, q, J = 2.4 Hz, 6-*H*), 4.46 (2H, brs, NH_2), 4.03 (3H, s, 17-OCH_3), 3.45 (3H, s, 6-OCH_3), 3.35 (3H, s, 12-OCH_3), 3.29 (2H, m, 11-*H*, 12-*H*), 2.85 (1H, m, 10-*H*), 2.26–2.41 (4H, m, 15-*H*, 4-*H*), 1.90 (3H, s, 22-CH_3), 1.80 (2H, m, 5-*H*), 1.64 (3H, s, 23-CH_3), 1.25–1.29 (3H, m, 13-*H*, 14-*H*), 1.10 (3H, d, J = 6.9 Hz, 25-CH_3), 0.93 (3H, d, J = 6.9 Hz, 24-CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 184.30, 170.03, 156.45, 155.55, 140.64, 132.29 128.21, 110.76, 82.24, 81.66, 78.69, 77.42, 77.00, 76.57, 76.14, 59.84, 57.57, 31.70, 31.11, 29.37, 24.54, 22.05, 13.01, 12.27, 12.19; MS(ESI) m/z 628 $(M + Na)^+$, 604 $(M - H)^-$; HRMS (EI) m/z calcd for $C_{30}H_{43}N_3O_{10}$ $[M^+]$ 605.2948, found: 605.2949; purity 96% (as determined by RP-HPLC, method A, R_t = 16.8 min; method B, R_t = 15.3 min).

General procedure for the synthesis of 11-carbamate-17-alkylamino-4,5-dihydrogeldanamycin derivatives 7a–d

To a solution of **6** (0.11 mmol, 1.0 equiv.) in DCE (5.0 mL) was added the appropriate amine (0.35 mL, 2 M solution in THF, 3.5 equiv.) and the reaction was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate and washed with aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. Purification by preparative TLC (CH_2Cl_2–MeOH = 20 : 1) gave **7a–d** as purple solids.

11-Carbamate-17-(ethylamino)-4,5-dihydro-17-demethoxygeldanamycin 7a

24.3 mg, 36% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.28 (1H, s, N*H*), 7.09 (1H, s, 19-*H*), 6.46 (1H, t, J = 6.6 Hz, 3-*H*), 5.34 (1H, d, J = 9.6 Hz, 9-*H*), 4.96 (1H, d, J = 6.6 Hz, 7-*H*), 4.76 (2H, brs, NH_2), 4.5–4.62 (3H, m, NH_2, 6-*H*), 3.45–3.53 (3H, m, CH_3CH_2NH, 11-*H*), 3.43 (3H, s, 6-OCH_3), 3.39 (3H, s, 12-OCH_3), 3.20 (1H, m, 12-*H*), 2.75–2.82 (1H, m, 10-*H*), 2.32–2.38 (4H, m, 4-*H*, 15-*H*), 1.90 (3H, s, 22-CH_3), 1.74–1.35 (5H, m, 5-*H*, 13-*H*, 14-*H*), 1.54 (3H, s, 23-CH_3), 1.30 (3H, m, CH_3CH_2NH), 1.04 (3H, s, 25-CH_3), 1.02 (3H, s, 24-CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 184.21, 180.73, 169.06, 156.03, 155.73, 144.24, 142.54, 133.82, 132.53, 131.15, 111.86, 107.66, 82.49, 80.63, 79.19, 78.11, 77.43, 77.00, 76.58, 59.86, 58.20, 39.80, 35.62, 31.94, 31.05, 30.62, 29.69, 25.07, 20.80, 15.57, 14.04, 12.26, 12.08; MS(ESI) m/z 641 $(M + Na)^+$, 617 $(M - H)^-$; HRMS (EI) m/z calcd for $C_{31}H_{46}N_4O_9$ $[M^+]$ 618.3265, found: 618.3265; purity >99% (as determined by RP-HPLC, method A, R_t = 15.8 min; method B, R_t = 6.7 min).

11-Carbamate-17-(allylamino)-4,5-dihydro-17-demethoxygeldanamycin 7b

28.9 mg, 27.0% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.24 (1H, s, N*H*), 7.12 (1H, s, 19-*H*), 6.46 (1H, t, J = 6.6 Hz, 3-*H*), 5.84–5.97 (1H, m, CH_2=C*H*CH_2), 5.36–5.50 (3H, m, CH_2=CHCH_2, 9-*H*), 5.10 (1H, d, J = 7.5 Hz, 7-*H*), 4.89 (2H, brs, NH_2), 4.71–4.76 (3H, m, NH_2, 6-*H*), 4.20 (2H, d, J = 4.8 Hz, CH_2=CHCH_2NH), 3.61–3.68 (1H, m, 11-*H*), 3.57 (3H, s, 6-OCH_3), 3.54 (3H, s, 12-OCH_3), 3.32 (1H, m, 12-*H*), 2.93 (1H, m, 10-*H*), 2.40–2.52 (4H, m, 15-*H*, 4-*H*), 2.04 (3H, s, 22-CH_3), 1.68 (3H, s, 23-CH_3), 1.88–1.42 (5H, m, 5-*H*, 13-*H*, 14-*H*), 1.83 (3H, s, 25-CH_3), 1.61 (3H, s, 24-CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 184.15, 181.01, 169.02, 156.07, 155.75, 144.13, 142.44, 140.01, 133.42, 132.49, 131.11, 117.67, 111.84, 108.15, 107.48, 82.51, 80.57, 79.06, 78.28, 77.73, 77.00, 76.58, 59.85, 58.27, 46.96, 35.89, 31.96, 31.87, 30.92, 30.60, 29.67, 25.04, 20.69, 13.99, 12.25, 12.03; MS(ESI) m/z 653 $(M + Na)^+$, 629 $(M - H)^-$; HRMS (EI) m/z calcd for $C_{32}H_{46}N_4O_9$ $[M^+]$: 630.3265, found: 630.3264; purity >99% (as determined by RP-HPLC, method A, R_t = 7.64 min; method B, R_t = 15.4 min).

11-Carbamate-17-(2-(dimethylamino)ethylamino)-4,5-dihydro-17-demethoxygeldanamycin 7c

32.6 mg, 35% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.27 (1H, s, N*H*), 7.07 (1H, s, 19-*H*), 6.74 (1H, t, J = 4.8 Hz, N*H*), 6.45 (1H, t, J = 6.6 Hz, 3-*H*), 5.34 (1H, d, J = 9.6 Hz, 9-*H*), 4.95 (1H, d, J = 7.2 Hz, 7-*H*), 4.83 (2H, brs, NH_2), 4.70 (2H, brs, NH_2), 4.59 (1H, m, 6-*H*), 3.46–3.56 (3H, m, 11-*H*, $(CH_3)_2NCH_2CH_2$NH), 3.42 (3H, s, 6-OCH_3), 3.39 (3H, s, 12-OCH_3), 3.18 (1H, m, 12-*H*), 2.78 (1H, m, 10-*H*), 2.53–2.70 (4H, m, 4-*H*, $(CH_3)_2NCH_2CH_2$NH), 2.31 (8H, m, $(CH_3)_2$N, 15-*H*), 1.89 (3H, s, 22-CH_3), 1.74–1.32 (5H, m, 5-*H*, 13-*H*, 14-*H*), 1.53 (3H, s, 23-CH_3), 1.01–1.04 (6H, m, 24-CH_3, 25-CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 184.47, 180.34, 169.02, 156.09, 155.78, 144.92, 142.15, 139.83, 132.57, 131.12, 107.66, 82.47, 80.60, 79.19, 77.42, 77.00, 76.58, 59.86, 58.23, 44.91, 41.95, 35.70, 31.93, 30.61, 29.67, 25.04, 20.81, 14.08, 12.24, 12.07;

MS(ESI) m/z 684 $(M + H)^+$, 660 $(M - H)^-$; HRMS (EI) m/z calcd for $C_{33}H_{51}N_5O_9$ $[M^+]$: 661.3687, found: 661.3689; purity 96% (as determined by RP-HPLC, method A, $R_t = 16.8$ min; method B, $R_t = 15.4$ min).

11-Carbamate-17-(2-(diethylamino)ethylamino)-4,5-dihydro-17-demethoxygeldanamycin 7d

42.0 mg, 32% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.27 (1H, s, N*H*), 7.05 (1H, s, 19-*H*), 6.93 (1H, t, $J = 4.8$ Hz, N*H*), 6.45 (1H, t, $J = 6.6$ Hz, 3-*H*), 5.34 (1H, d, $J = 9.0$ Hz, 9-*H*), 4.94 (1H, d, $J = 7.2$ Hz, 7-*H*), 4.81 (2H, brs, NH_2), 4.67 (2H, brs, NH_2), 4.58 (1H, q, $J = 2.4$ Hz, 6-*H*), 3.49–3.56 (3H, m, 11-*H*, NHCH_2), 3.42 (3H, s, 6-OCH_3), 3.39 (3H, s, 12-OCH_3), 3.17 (1H, m, 12-*H*), 2.62–2.84 (7H, m, NHCH$_2$CH_2N(CH_2CH$_3$)$_2$, 10-*H*), 2.33 (4H, m, 4-*H*, 15-*H*), 1.89 (3H, s, 22-CH_3), 1.35–1.76 (5H, m, 5-*H*, 13-*H*, 14-*H*), 1.52 (3H, s, 23-CH_3), 1.01–1.08 (12H, m, N(CH$_2$CH_3)$_2$, 24-CH_3, 25-CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 179.90, 168.83 155.96, 155.65, 145.05, 141.96, 139.76, 132.46, 130.98, 107.63, 82.52, 80.54, 79.17, 78.34, 77.31, 77.20, 77.00, 76.68, 59.89, 58.34, 42.00, 31.98, 30.69, 29.75, 25.13, 20.85, 14.19 12.34, 12.12; MS(ESI) m/z 712 $(M + Na)^+$, 688 $(M - H)^-$; HRMS (EI) m/z calcd for $C_{35}H_{55}N_5O_9$ $[M^+]$ 689.4000, found: 689.4017; purity 96% (as determined by RP-HPLC, method A, $R_t = 8.4$ min; method B, $R_t = 6.4$ min).

3-Oxo-butyric acid 8,14,19-trimethoxy-4,10,12,16-tetramethyl-3,20,22-trioxo-13-(3-oxo-butyryloxy)-2-aza-bicyclo[16.3.1]docosa-1(21),4,10,18-tetraen-9-yl ester 8

To a solution of **4** (41.2 mg, 0.079 mmol), diketene (0.0067 mL, 0.087 mmol) and a catalytic amount of DMAP in THF (1 mL) was added Et_3N (0.0012 mL, 0.0087 mmol) slowly at room temperature. After stirring overnight, the mixture was concentrated. Purification by preparative TLC (*n*-hexane–EtOAc–MeOH = 6 : 3 : 1) gave **8** as a yellow solid (7.6 mg, 16% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.69 (1H, s, N*H*), 7.03 (1H, s, 19-*H*), 6.42 (1H, t, $J = 6.6$ Hz, 3-*H*), 5.28 (1H, d, $J = 9.9$ Hz, 9-*H*), 5.10 (1H, d, $J = 7.5$ Hz, 7-*H*), 4.75 (1H, q, $J = 2.7$ Hz, 6-*H*), 4.12 (3H, s, 17-OCH_3), 3.47 (2H, s, COCH_2CO), 3.41 (3H, s, 6-OCH_3), 3.37 (3H, s, 12-OCH_3), 3.35 (2H, s, COCH_2CO), 3.26 (2H, m, 11-*H*, 12-*H*), 2.87 (1H, m, 10-*H*), 2.35–2.41 (4H, m, 15-*H*, 4-*H*), 2.27 (3H, s, COCH_3), 2.20 (3H, s, COCH_3), 1.88 (3H, s, 22-CH_3), 1.59 (3H, s, 23-CH_3), 1.44 (2H, m, 5-*H*), 1.23–1.33 (3H, m, 13-*H*, 14-*H*), 1.10 (3H, d, $J = 6.9$ Hz, 25-CH_3), 0.97 (3H, d, $J = 6.6$ Hz, 24-CH_3); MS(ESI) m/z 710 $(M + Na)^+$, 686 $(M - H)^-$; HRMS (EI) m/z calcd for $C_{36}H_{49}NO_{12}$ $[M^+]$ 687.3255, found: 687.3257, purity 96% (as determined by RP-HPLC, method A, $R_t = 22.0$ min; method B, $R_t = 20.6$ min).

Methyl-carbamic acid 8,14-dimethoxy-4,10,12,16-tetramethyl-19-methylamino-13-methylcarbamoyloxy-3,20,22-trioxo-2-aza-bicyclo[16.3.1]docosa-1(21),4,10,18-tetraen-9-yl ester 9

To a stirred solution of **4** (40.0 mg, 0.077 mmol) in CH_2Cl_2 (1.0 mL) was added 1,1′-carbonyldiimidazole (31.2 mg, 0.19 mmol). After stirring overnight at room temperature, methylamine (0.014 mL, 2 M solution in THF) was added, and the mixture was stirred for an additional 1 h. The reaction mixture was concentrated, and purification by preparative TLC (*n*-hexane–EtOAc–MeOH = 6 : 3 : 1) gave **9** as a purple solid (7.4 mg, 15% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.44 (1H, s, N*H*), 7.11 (1H, s, 19-*H*), 6.54 (1H, m, 3-*H*), 5.30 (1H, d, $J = 9.3$ Hz, 9-*H*), 4.95 (1H, d, $J = 7.5$ Hz, 7-*H*), 4.62 (1H, m, 6-*H*), 3.52 (1H, m, 11-*H*), 3.36 (6H, s, 6-OCH_3, NHCH_3), 3.34 (1H, m, 12-*H*), 3.16 (3H, s, 12-OCH_3), 2.71–2.74 (7H, m, CONHCH_3, 10-*H*), 2.35–2.41 (4H, m, 15-*H*, 4-*H*), 2.00 (3H, s, 22-CH_3), 1.86 (3H, s, 23-CH_3), 1.45–1.78 (5H, m, 5-*H*, 13-*H*, 14-*H*), 1.21 (3H, t, $J = 6.9$ Hz, 25-CH_3), 1.00 (3H, t, $J = 6.6$ Hz, 24-CH_3); MS(ESI) m/z 655 $(M + H)^+$, 631 $(M - H)^-$; HRMS (EI) m/z calcd for $C_{32}H_{48}N_4O_9$ $[M^+]$ 632.3421, found: 632.3423; purity 97% (as determined by RP-HPLC, method A, $R_t = 17.5$ min; method B, $R_t = 16.1$ min).

General procedure for the synthesis of 17-alkylamino-4,5-dihydrogeldanamycin derivatives 10a–g

To a solution of 4,5-dihydrogeldanamycin **5** (0.13 mmol, 1.0 equiv.) in DCE (5.0 mL) was added the appropriate amine (0.26 mmol, 2.0 equiv.) and the reaction was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate and washed with aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by preparative TLC (CH_2Cl_2–MeOH = 20 : 1) gave **10a–g** as purple solids.

17-(Ethylamino)-4,5-dihydro-17-demethoxygeldanamycin 10a

45.2 mg, 62% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.30 (1H, s, N*H*), 7.14 (1H, s, 19-*H*), 6.23–6.25 (2H, m, 3-*H*, N*H*), 5.79 (1H, d, $J = 9.6$ Hz, 9-*H*), 5.20 (1H, d, $J = 5.2$ Hz, 7-*H*), 4.68 (2H, brs, NH_2), 3.41 (3H, s, 6-OCH_3), 3.37 (3H, s, 12-OCH_3), 3.33–3.62 (5H, m, 6-*H*, 11-*H*, 12-*H*, CH_3CH_2NH), 2.72 (1H, m, 10-*H*), 2.41 (4H, m, 4-*H*, 15-*H*), 1.91 (3H, s, 22-CH_3), 1.64–1.76 (5H, m, 5-*H*, 13-*H*, 14-*H*), 1.68 (3H, s, 23-CH_3), 1.34 (3H, t, $J = 6.8$ Hz, CH_3CH$_2$NH), 1.02 (3H, d, $J = 7.2$ Hz, 25-CH_3), 0.97 (3H, d, $J = 6.8$ Hz, 24-CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 183.76, 180.77, 168.24, 156.11, 144.67, 141.60, 137.96, 135.09, 133.13, 130.45, 108.18, 82.05, 81.59, 80.95, 77.43, 77.00, 76.58, 73.26, 57.08, 40.52, 35.25, 32.77, 30.17, 29.69, 29.08 24.32, 22.46, 15.17, 12.81, 12.19; MS(ESI) m/z 598 $(M + Na)^+$, 574 $(M - H)^-$; HRMS (EI) m/z calcd for $C_{30}H_{45}N_3O_8$ $[M^+]$ 575.3207, found: 575.3204; purity >99% (as determined by RP-HPLC, method A, $R_t = 17.2$ min; method B, $R_t = 15.8$ min).

17-(Allylamino)-4,5-dihydro-17-demethoxygeldanamycin 10b

44.9 mg, 55% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.23 (1H, s, N*H*), 7.12 (1H, s, 19-*H*), 6.39 (1H, t, $J = 6.0$ Hz, N*H*), 6.21 (1H, m, 3-*H*), 5.90 (1H, m, CH_2=C*H*), 5.75 (1H, d, $J = 9.0$ Hz, 9-*H*), 5.27 (2H, m, CH_2=CH), 5.15 (1H, d, $J = 5.7$ Hz, 7-*H*), 4.91 (2H, brs, NH_2), 4.11 (2H, t, $J = 6.3$ Hz, CH_2=CHCH_2NH), 3.57 (1H, m, 6-*H*), 3.28–3.44 (2H, m, 11-*H*, 12-*H*), 3.38 (3H, s, 6-OCH_3), 3.34 (3H, s, 12-OCH_3), 2.62–2.67 (1H, m, 10-*H*), 2.26–2.40 (4H, m, 4-*H*, 15-*H*), 1.88 (3H, s, 22-CH_3), 1.68 (5H, m, 5-*H*, 13-*H*, 14-*H*), 1.65 (3H, s, 23-CH_3), 0.95–0.99 (6H, m, 24-CH_3, 25-CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 183.72, 181.09, 168.23, 156.12, 144.57, 141.43, 138.06, 135.04, 133.11, 132.65, 130.45, 118.36, 108.64, 107.70, 82.01, 81.48, 80.96, 77.42, 77.00, 76.57, 73.39, 59.01, 57.08, 47.62, 35.29, 33.80, 32.75, 30.17, 29.68, 29.12, 24.34, 22.41, 12.82, 12.28, 12.20; MS(ESI) m/z 610 $(M + Na)^+$, 586 $(M - H)^-$; HRMS (EI) m/z calcd for $C_{31}H_{45}N_3O_8$ $[M^+]$ 587.3207,

found: 587.3207; purity >99% (as determined by RP-HPLC, method A, $R_t = 17.4$ min; method B, $R_t = 16.1$ min).

17-(2-(Dimethylamino)ethylamino)-4,5-dihydro-17-demethoxygeldanamycin 10c

18.9 mg, 20% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.26 (1H, s, N*H*), 7.09 (1H, s, 19-*H*), 7.00 (1H, brs, N*H*), 6.23 (1H, t, $J = 6.6$ Hz 3-*H*), 5.78 (1H, d, $J = 9.3$ Hz, 9-*H*), 5.17 (1H, d, $J = 5.4$ Hz, 7-*H*), 4.80 (2H, brs, NH_2), 3.82 (1H, m, 6-*H*), 3.30–3.72 (4H, m, 11-*H*, 12-*H*, $(CH_3)_2NCH_2CH_2NH$), 3.39 (3H, s, 6-OCH_3), 3.34 (3H, s, 12-OCH_3), 2.66–2.75 (3H, m, 10-*H*, $(CH_3)_2NCH_2CH_2NH$), 2.34–2.41 (10H, s, $(CH_3)_2$N, 4-*H*, 15-*H*), 1.89 (3H, s, 22-CH_3), 1.72–1.69 (5H, m, 5-*H*, 13-*H*, 14-*H*), 1.67 (3H, s, 23-CH_3), 0.95–1.00 (6H, m, 24-CH_3, 25-CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 168.11, 155.98, 137.71, 134.95, 133.09, 130.45, 107.89, 107.35, 82.20, 81.57, 80.92, 77.32, 77.21, 77.00, 76.69, 73.20, 59.04, 57.07, 44.87, 35.31, 32.82, 31.27, 30.27, 29.05, 24.37, 22.64, 12.87, 12.40, 12.31; MS(ESI) m/z 619 $(M + H)^+$, 617 $(M - H)^-$; HRMS (EI) m/z calcd for $C_{32}H_{50}N_4O_8$ $[M^+]$ 618.3629, found: 618.3634; purity >99% (as determined by RP-HPLC, method A, $R_t = 7.9$ min; method B, $R_t = 5.8$ min).

17-(2-(Diethylamino)ethylamino)-4,5-dihydro-17-demethoxygeldanamycin 10d

46.8 mg, 52% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.24 (1H, s, N*H*), 7.18 (1H, t, $J = 5.1$ Hz, N*H*), 7.05 (1H, s, 19-*H*), 6.22 (1H, t, $J = 6.6$ Hz, 3-*H*), 5.75 (1H, d, $J = 9.0$ Hz, 9-*H*), 5.15 (1H, d, $J = 5.7$ Hz, 7-*H*), 4.92 (2H, brs, NH_2), 3.71 (2H, m, NHCH_2CH$_2$), 3.58–3.28 (3H, m, 6-*H*, 11-*H*, 12-*H*), 3.38 (3H, s, 6-OCH_3), 3.34 (3H, s, 12-OCH_3), 2.62–2.84 (7H, m, NHCH$_2$CH_2N(CH_2CH$_3$)$_2$, 10-*H*), 2.37 (4H, m, 4-*H*, 15-*H*), 1.88 (3H, s, 22-CH_3), 1.70 (5H, m, 5-*H*, 13-*H*, 14-*H*), 1.65 (3H, s, 23-CH_3), 1.12 (6H, m, N(CH$_2$CH_3)$_2$), 0.95–0.99 (6H, m, 24-CH_3, 25-CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 180.08, 168.27, 156.20, 137.85, 135.10, 133.16, 130.49, 82.19, 81.54, 80.98, 77.32, 77.00, 76.68, 73.28, 59.04, 57.07, 35.30, 32.73, 30.22, 29.64, 29.28, 28.96, 24.28, 22.64, 22.51, 12.75, 12.29, 12.20; MS(ESI) m/z 647 $(M + H)^+$, 645 $(M - H)^-$; HRMS (EI) m/z calcd for $C_{34}H_{54}N_4O_8$ $[M^+]$ 646.3942, found: 646.3947; purity >99% (as determined by RP-HPLC, method A, $R_t = 8.7$ min; method B, $R_t = 6.6$ min).

17-(2-(Pyrrolidin-1-yl)ethylamino)-4,5-dihydro-17-demethoxygeldanamycin 10e

10.9 mg, 12% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.21 (1H, s, N*H*), 7.07 (1H, s, 19-*H*), 6.97 (1H, brs, N*H*), 6.23 (1H, t, $J = 6.6$ Hz, 3-*H*), 5.77 (1H, d, $J = 9.3$ Hz, 9-*H*), 5.17 (1H, d, $J = 4.8$ Hz, 7-*H*), 4.82 (2H, brs, NH_2), 4.74 (1H, brs, O*H*), 3.56–3.85 (4H, m, NHCH_2CH$_2$N, 6-*H*, 11-*H*), 3.45 (1H, m, 12-*H*), 3.39 (3H, s, 6-OCH_3), 3.35 (3H, s, 12-OCH_3), 2.91–3.04 (6H, m, NHCH$_2$CH_2N, pyrrolidine-*H*), 2.63–2.68 (1H, m, 10-*H*), 2.37 (4H, m, 4-*H*, 15-*H*), 2.02–2.06 (4H, m, pyrrolidine-*H*), 1.89 (3H, s, 22-CH_3), 1.67–1.72 (8H, m, 23-CH_3, 5-*H*, 13-*H*, 14-*H*), 0.98–1.00 (6H, m, 24-CH_3, 25-CH_3); MS(ESI) m/z 667 $(M + Na)^+$, 643 $(M - H)^-$; HRMS (EI) m/z calcd for $C_{34}H_{52}N_4O_8$ $[M^+]$ 644.3785, found: 644.3783; purity >99% (as determined by RP-HPLC, method A, $R_t = 8.4$ min; method B, $R_t = 6.3$ min).

17-(2-Morpholinoethylamino)-4,5-dihydro-17-demethoxygeldanamycin 10f

50.7 mg, 55% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.25 (1H, s, N*H*), 7.08 (1H, s, 19-*H*), 7.06 (1H, brs, N*H*), 6.23 (1H, t, $J = 6.6$ Hz, 3-*H*), 5.77 (1H, d, $J = 9.6$ Hz, 9-*H*), 5.16 (1H, d, $J = 5.4$ Hz, 7-*H*), 4.88 (2H, brs, NH_2), 3.42–3.76 (9H, m, NHCH_2CH$_2$, morpholine-*H*, 6-*H*, 11-*H*, 12-*H*), 3.38 (3H, s, 6-OCH_3), 3.34 (3H, s, 12-OCH_3), 2.51–2.74 (7H, m, NHCH$_2$CH_2, morpholine-*H*, 10-*H*), 2.36–2.39 (4H, m, 4-*H*, 15-*H*), 1.88 (3H, s, 22-CH_3), 1.71 (5H, m, 5-*H*, 13-*H*, 14-*H*), 1.66 (3H, s, 23-CH_3), 0.94–0.99 (6H, m, 24-CH_3, 25-CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 180.44, 168.25, 141.31, 137.87, 134.96, 133.13, 130.53, 107.82, 82.16, 81.54, 80.90, 77.42, 77.00, 76.57, 73.21, 59.00, 57.00, 52.91, 35.25, 33.95, 32.74, 30.16, 28.98, 24.32, 22.63, 12.77, 12.27, 12.19; MS(ESI) m/z 683 $(M + Na)^+$, 659 $(M - H)^-$; HRMS (EI) m/z calcd for $C_{34}H_{52}N_4O_9$ $[M^+]$ 660.3734, found: 660.3736; purity 99% (as determined by RP-HPLC, method A, $R_t = 8.0$ min; method B, $R_t = 5.9$ min).

17-(2-(1*H*-Imidazol-1-yl)propylamino)-4,5-dihydro-17-demethoxygeldanamycin 10g

45.8 mg, 54% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.19 (1H, s, N*H*), 7.09 (1H, s, 19-*H*), 6.25 (1H, t, $J = 6.3$ Hz, N=C*H*N), 6.19 (1H, t, $J = 6.9$ Hz, 3-*H*), 5.72 (1H, d, $J = 9.3$ Hz, 9-*H*), 5.12 (1H, d, $J = 4.8$ Hz, 7-*H*), 4.94 (2H, brs, NH_2), 4.11 (2H, m, NC*H*=C*H*N), 3.41–3.62 (7H, m, NHCH_2CH$_2$CH_2, 6, 11, 12), 3.37 (3H, s, 6-OCH_3), 3.33 (3H, s, 12-OCH_3), 2.68 (1H, m, 10-*H*), 2.34 (2H, m, NHCH$_2$CH_2CH$_2$), 2.15 (4H, m, 4-*H*, 15-*H*), 1.87 (3H, s, 22-CH_3), 1.53–1.68 (8H, s, 23-CH_3, 5-*H*, 13-*H*, 14-*H*), 0.96 (3H, d, $J = 6.6$ Hz, 24-CH_3), 0.85 (3H, d, $J = 5.7$ Hz, 25-CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 183.71, 181.13, 168.24, 156.19, 144.32, 141.39, 138.22, 134.90, 133.03, 130.53, 109.15, 107.66, 81.99, 81.32, 80.93, 77.43, 77.00, 76.58, 73.25, 58.99, 57.12, 42.28, 35.08, 33.82, 32.70, 31.01, 30.07, 29.66, 29.05, 24.34, 22.37, 12.79, 12.27, 12.20; MS(ESI) m/z 678 $(M + Na)^+$, 654 $(M - H)^-$; HRMS (EI) m/z calcd for $C_{34}H_{49}N_5O_8$ $[M^+]$ 655.3581, found: 655.3583; purity >99% (as determined by RP-HPLC, method A, $R_t = 8.6$ min; method B, $R_t = 6.5$ min).

11-Carbamategeldanamycin 11

To a stirred solution of geldanamycin **1** (253.9 mg, 0.45 mmol) in CH_2Cl_2 (9.0 mL) was added trichloroacetyl isocyanate (0.067 mL, 0.57 mmol) at 0 °C and the reaction was stirred at room temperature for 3 h. CH_2Cl_2 and an excess amount of Al_2O_3 were added to the reaction mixture and the resulting suspension was stirred overnight and filtered. The filtrate was concentrated to give **11** as a yellow solid (267.6 mg, 98.5% yield), which was used as such in the next step without further purification. $R_f = 0.26$ (CH_2Cl_2–MeOH = 20 : 1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.61 (1H, s, N*H*), 7.17 (1H, s, 19-*H*), 6.56 (1H, t, $J = 11.7$ Hz, 3-*H*), 5.81 (1H, t, $J = 10.5$ Hz, 4-*H*), 5.19 (1H, d, $J = 10.2$ Hz, 5-*H*), 5.81 (1H, s, 9-*H*), 4.62 (1H, d, $J = 7.5$ Hz, 7-*H*), 4.40 (1H, d, $J = 9.9$ Hz, 6-*H*), 4.07 (1H, m, 11-*H*), 4.03 (3H, s, 17-OCH_3), 3.37 (3H, s, 6-OCH_3), 3.35 (3H, s, 12-OCH_3), 3.31 (1H, m, 12-*H*), 2.95 (1H, m, 10-*H*), 2.23–2.47 (2H, m, 15-*H*), 2.02 (3H, s, 22-CH_3), 1.90 (1H, m, 14-*H*), 1.77 (3H, s, 23-CH_3), 1.51–1.65 (2H, m, 13-*H*), 1.14 (3H, d, $J = 6.9$ Hz, 25-CH_3), 0.88 (3H, d, $J = 7.2$ Hz, 24-CH_3); MS(ESI) m/z 628 $(M + Na)^+$, 602 $(M - H)^-$.

11-Carbamate-17-(allylamino)-17-demethoxygeldanamycin 12a

To a stirred solution of 17-AAG **2** (50.5 mg, 0.086 mmol) in CH_2Cl_2 (2.0 mL) was added trichloroacetyl isocyanate (0.013 mL, 0.108 mmol) at 0 °C and the resulting solution was stirred at room temperature for 1.5 h. To the reaction mixture were added CH_2Cl_2 and an excess amount of Al_2O_3. The mixture was stirred for 2 h and filtered. The filtrate was concentrated. Purification by preparative TLC (CH_2Cl–MeOH = 10 : 1) gave **12a** as a purple solid (43.5 mg, 80% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.23 (1H, s, N*H*), 7.15 (1H, s, 19-*H*), 6.54 (1H, ps-t, J = 11.7 Hz, 3-*H*), 5.90 (2H, m, 4-*H*, CH_2=C*H*CH_2), 5.24–5.33 (2H, m, 5-*H*, 9-*H*), 4.80 (3H, m, 7-*H*, C*H*$_2$=CHCH_2NH), 4.48 (1H, d, J = 8.1 Hz, 6-*H*), 4.43 (2H, brs, N*H*$_2$), 4.09 (2H, d, J = 5.4 Hz, CH_2=CHC*H*$_2$NH), 3.50 (1H, m, 11-*H*), 3.36 (3H, s, 6-OC*H*$_3$), 3.24–3.38 (4H, s, 12-OC*H*$_3$, 12-*H*), 2.87–2.94 (1H, m, 10-*H*), 2.56–2.63 (1H, m, 15-*H*), 2.27–2.34 (1H, m, 15-*H*), 2.01 (3H, s, 22-C*H*$_3$), 1.81–1.86 (1H, m, 14-*H*), 1.74 (3H, s, 23-C*H*$_3$), 1.52–1.61 (2H, m, 13-*H*), 1.03 (3H, d, J = 6.6 Hz, 25-C*H*$_3$), 0.97 (3H, d, J = 7.5 Hz, 24-C*H*$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 184.27, 180.35, 167.56, 155.88, 144.35, 133.14, 118.00, 111.85, 108.62, 107.44, 79.53, 77.42, 77.00, 76.58, 47.66, 32.31, 28.14, 13.75, 12.33; MS(ESI) m/z 651 (M + Na)$^+$, 627 (M − H)$^-$; HRMS (EI) m/z calcd for $C_{32}H_{44}N_4O_9$ [M$^+$] 628.3108, found: 628.3099; purity >99% (as determined by RP-HPLC, method A, R_t = 16.9 min; method B, R_t = 15.6 min).

General procedure for the synthesis of 11-carbamate-17-alkylamino derivatives of geldanamycin 12b–d

To a solution of 11-carbamategeldanamycin **11** (0.11 mmol, 1.0 equiv.) in DCE (5.0 mL) was added the appropriate amine (0.33 mmol, 3.0 equiv.) and the reaction was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate and washed with aqueous sodium bicarbonate and brine. The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by preparative TLC (CH_2Cl_2–MeOH = 20 : 1) gave **12b–d** as purple solids.

11-Carbamate-17-(2-(dimethylamino)ethylamino)-17-demethoxygeldanamycin 12b

21.7 mg, 30% yield. ^{1}H NMR (CD_3OD + $CDCl_3$, 300 MHz) δ 7.24 (1H, brd, J = 11.4 Hz, 3-*H*), 6.98 (1H, s, 19-*H*), 6.56 (1H, ps-t, J = 11.4 Hz, 4-*H*), 5.84 (1H, ps-t, J = 10.5 Hz, 5-*H*), 5.30 (2H, m, 9-*H*, 7-*H*), 4.59 (1H, d, J = 7.8 Hz, 6-*H*), 3.68 (2H, m, $(CH_3)_2NCH_2CH_2NH$), 3.60 (2H, m, 11-*H*, 12-*H*), 3.30 (6H, s, 6-OC*H*$_3$, 12-OC*H*$_3$), 2.83–2.94 (3H, m, 10-*H*, $(CH_3)_2NCH_2CH_2NH$), 2.50 (6H, s, $(CH_3)_2$N), 2.23–2.00 (2H, m, 15-*H*), 1.98 (3H, s, 22-C*H*$_3$), 1.75–1.88 (1H, m, 14-*H*), 1.69 (3H, s, 23-C*H*$_3$), 1.31–1.63 (2H, m, 13-*H*), 1.02 (3H, d, J = 6.9 Hz, 25-C*H*$_3$), 0.97 (3H, d, J = 6.6 Hz, 24-C*H*$_3$); MS(ESI) m/z 682 (M + Na)$^+$, 658 (M − H)$^-$; HRMS (EI) m/z calcd for $C_{33}H_{49}N_5O_9$ [M$^+$] 659.353; found: 659.3531; purity 99% (as determined by RP-HPLC, method A, R_t = 7.9 min; method B, R_t = 5.7 min).

11-Carbamate-17-(2-(diethylamino)ethylamino)-17-demethoxygeldanamycin 12c

10.4 mg, 12% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.22 (1H, s, N*H*), 7.07 (1H, s, 19-*H*), 6.53 (1H, ps-t, J = 11.4 Hz, 3-*H*), 5.82 (1H, t, J = 10.2 Hz, 4-*H*), 5.30 (2H, m, 5-*H*, 9-*H*), 4.80 (3H, brs, 7-*H*, N*H*$_2$), 4.46 (1H, d, J = 7.8 Hz, 6-*H*), 3.59–3.76 (3H, m, 11-*H*, NHC*H*$_2$), 3.48 (1H, m, 12-*H*), 3.35 (3H, s, 12-OC*H*$_3$), 3.34 (3H, s, 6-OC*H*$_3$), 2.76–2.89 (7H, m, 10-*H*, $NHCH_2CH_2N(CH_2CH_3)_2$), 2.31–2.34 (2H, m, 15-*H*), 2.00 (3H, s, 22-C*H*$_3$), 1.87 (1H, m, 14-*H*), 1.74 (3H, s, 23-C*H*$_3$), 1.36–1.60 (2H, m, 13-*H*), 1.17–1.19 (6H, m, $N(CH_2CH_3)_2$), 1.05 (3H, d, J = 6.0 Hz, 25-C*H*$_3$), 0.95 (3H, d, J = 6.0 Hz, 24-C*H*$_3$); MS(ESI) m/z 710 (M + Na)$^+$, 686 (M − H)$^-$; HRMS (EI) m/z calcd for $C_{35}H_{53}N_5O_9$ [M$^+$] 687.3843, found: 687.3854; purity 97% (as determined by RP-HPLC, method A, R_t = 9.1 min; method B, R_t = 7.1 min).

11-Carbamate-17-(2-(pyrrolidin-1-yl)ethylamino)-17-demethoxygeldanamycin 12d

17.0 mg, 35% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.23 (1H, s, N*H*), 7.08 (1H, s, 19-*H*), 6.69 (1H, brs, 3-*H*), 6.53 (1H, ps-t, J = 11.4 Hz, 4-*H*), 5.82 (1H, t, J = 10.2 Hz, 5-*H*), 5.30 (2H, m, 9-*H*, 7-*H*), 4.82 (2H, brs, N*H*$_2$), 4.47 (1H, d, J = 8.4 Hz, 6-*H*), 3.67 (2H, m, NHC*H*$_2$), 3.48 (2H, m, 11-*H*, 12-*H*), 3.46 (6H, s, 6-OC*H*$_3$, 12-OC*H*$_3$), 2.60–2.90 (7H, m, $NHCH_2CH_2$-, pyrrolidine-*H*, 10-*H*), 2.02–2.40 (2H, m, 15-*H*), 2.00 (3H, s, 22-C*H*$_3$), 1.79–1.89 (5H, m, pyrrolidine-*H*, 14-*H*), 1.73 (3H, s, 23-C*H*$_3$), 1.42–1.68 (2H, m, 13-*H*), 1.08 (3H, d, J = 6.9 Hz, 25-C*H*$_3$), 0.94 (3H, d, J = 6.6 Hz, 24-C*H*$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 185.64, 179.79, 169.98, 156.33, 145.85, 141.27, 134.44, 133.20, 126.58, 113.02, 111.85, 108.33, 79.63, 77.43, 77.00, 76.58, 75.85, 57.39, 56.83, 55.15, 41.94, 32.37, 23.21, 13.48, 12.32; MS(ESI) m/z 686 (M + H)$^+$, 684 (M − H)$^-$; HRMS (EI) m/z calcd for $C_{35}H_{51}N_5O_9$ [M$^+$] 685.3687, found: 685.3683; purity 97% (as determined by RP-HPLC, method A, R_t = 8.8 min; method B, R_t = 6.7 min).

Biological procedures

MTT assays

To investigate the cell growth inhibition activity of the compounds, we performed MTT assays. In brief, exponentially growing cells were seeded into a 96-well plate and incubated. After 24 hours, drugs were added at given concentrations, followed by incubation for 48 hours. MTT was given to the wells at a concentration of 0.65 mg ml^{-1}, and the cells were incubated for 4 hours. The formazan was solubilized by a lysis buffer (10% SDS in 0.1 N HCl), and the absorbance read at 570–650 nm with a microplate reader (Quant, Bio-Tek, USA). The cell viabilities of each well were compared with a blank.

TGI assays

Cells were seeded into two 96-well plates at 1 × 10^4 cells per well and incubated for 24 hours. Before adding the drugs, we performed an MTT assay with one plate (T0). Another plate treated with compounds at various concentrations was incubated for another 24 hours and underwent MTT assays (T1). With these measurements TGI values of the drugs were calculated (the concentration where T0 = T1).

Western blot analysis

After incubation with the drugs, the cells were harvested, washed twice in PBS, and lysed in lysis buffer [50 mM Tris-Cl (pH 7.4), 1%

triton X-100, 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, and a cocktail of protease inhibitors (Sigma, St Louis, MO, USA)]. Lysates (30 μg) were loaded onto 8% SDS-PAGE and transferred to a PVDF membrane. The blot was incubated overnight with an antibody to ErbB2 from Cell Signaling Technology Inc., followed by goat anti-rabbit antibody coupled to peroxidase antibody. The bound antibody was visualized by ECL (Amersham Biosciences, Buckinghamshire, England).

Conclusions

A new series of potent geldanamycin derivatives were prepared *via* structural modifications of geldanamycin, 4,5-dihydrogeldanamycin, and 4,5-dihydro-7-*O*-descarbamoyl-7-hydroxygeldanamycin. Most of the synthesized compounds exhibited potent *in vitro* anti-proliferation activity in human cancer cell lines, SK-Br3 and SK-Ov3. To confirm their anticancer properties *via* Hsp90 inhibition, the selected compounds **7d**, **12b**, and **12d** were further evaluated in terms of their potential to inhibit the expression of ErbB2, one of the client proteins of Hsp90, in SK-Ov3 cell line. In the present study, compound **12b**, at the TGI concentration of 17 μM, was shown to remarkably reduce ErbB2 levels within 4 h. Additional studies related to these analogues are in progress and will be reported in due course.

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