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Improved Synthesis and Evaluation of 17-Substituted Aminoalkylgeldanamycin Derivatives Applicable to Drug Delivery Systems

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Abstract—The 17-methoxy group of geldanamycin was substituted with 1,3-diaminopropane and 1,3-diamino-2-hydroxypropane to introduce a primary amino group useful for conjugation with targeting moieties and drug carriers. We have developed a procedure that has provided improved yield and reproducibility of the syntheses. Both geldanamycin derivatives demonstrated antiproliferative activity towards the human ovarian carcinoma cell line, A2780.

Geldanamycin (GDM) is a benzoquinone ansamycin antibiotic with anticancer activity.[1,2] It binds to heat shock proteins such as HSP90[3] and GRP94,[4] inhibiting their capacity to form complexes with client oncoproteins.[3–7] This results in the degradation and inhibition of the oncoproteins. However, the development of GDM as a new anticancer drug has been impaired due to its severe toxicity.[8] To reduce GDM's side effects, several drug delivery systems (DDS) have been proposed.[9–12]

Since GDM (compound **1** in Fig. 1) does not possess functional groups suitable for conjugation with targeting moieties and drug carriers, the methoxy group at the 17-position has been substituted with several diaminoalkanes.[9,10,12] Modification of the 17-position is widely acceptable[13] and the generated primary amino group is useful for the conjugation. 17-(3-Aminopropylamino)-17-demethoxygeldanamycin (AP-GDM, compound **2a**) has often been applied to DDS due to the maintenance of drug activity and its relatively low hydrophobicity.[10,12]

Difficulty in the synthesis of AP-GDM was revealed due to the conversion of AP-GDM to 17,18-diazepino-17-demethoxygeldanamycin as a result of the formation of an intramolecular imine linkage (Fig. 1).[9,13] This reaction was facilitated in the presence of silica gel, and interfered with the purification of the product. Also, we found that the use of strong alkaline solutions for purification resulted in the loss of material, apparently due to the same reaction as suggested by the color changes from purple to brown. We hypothesized that protection of the 3′-amino group by protonation would suppress the side reaction. In this report, we describe a synthetic procedure that produces AP-GDM as a hydrochloride with high purity and good reproducibility. Furthermore, we synthesized and evaluated 17-(3-amino-2-hydroxypropylamino)-17-demethoxygeldanamycin (AP(OH)-GDM) as a novel geldanamycin derivative that is also applicable to DDS.

We found that AP-GDM·HCl (**3a**) was insoluble both in chloroform and in NaCl-saturated water, whereas GDM was soluble in chloroform and 1,3-diaminopropane in NaCl-saturated water. Since AP-GDM·HCl is soluble in aqueous solutions with low NaCl concentrations, its precipitation in NaCl-saturated water is attributable to the salting-out phenomenon. Based on this property, we isolated AP-GDM·HCl from the reaction mixture.[15] After the reaction in chloroform, the mixture was poured into NaCl-saturated water, and 6 M HCl was added with vigorous stirring of aqueous and organic layers for neutralization. This resulted in the precipitation of AP-GDM·HCl, which could be collected by filtration with contaminating NaCl. AP-GDM

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was extracted with chloroform as a free base from a weakly alkaline solution, leaving contaminating NaCl in the aqueous layer. After chloroform was evaporated and the residue redissolved in MeOH, AP-GDM was protonated with HCl, followed by precipitation with MeOH–ether. The reaction was repeated three times and the resulting yields were between 74 and 94%. These values are significantly higher than those previously reported.[13]

Previous literature has described that two equivalents of diaminopropane were introduced to GDM when an excess of diaminopropane was used, possibly as a result of 17-substitution and 18-imine formation with two separate diamines [formation of di(aminopropylamino) GDM derivative].[9] In contrast, diaminohexane can be selectively introduced to the 17 position even if a large excess of diaminohexane is used to suppress the cross-linking of two GDM molecules, according to another report.[14] In agreement with the latter case, here it was found that diaminopropane was selectively introduced into the 17 position when an excess of diaminopropane was used. However, when the reaction time was prolonged from 2 to 12 h, the yield of AP-GDM significantly decreased (data not shown), suggesting the probable formation of di(aminopropylamino) GDM derivative[9] as a side product. Although the mechanism of formation/suppression of the di(aminopropylamino) GDM derivative is not clear, it may be concluded that use of excess diaminopropane with a relatively short reaction time is favorable for the synthesis of AP-GDM in our system. We have confirmed that the 3′-amino group on AP-GDM is reactive toward *p*-nitrophenol active ester in the presence of weak base such as 4-dimethylaminopyridine.[12] This indicates that the aminolysis of the active ester is faster than the intramolecular imine formation, validating the utility of AP-GDM·HCl for further modification in the case of attachment to targeting moieties and drug carriers.

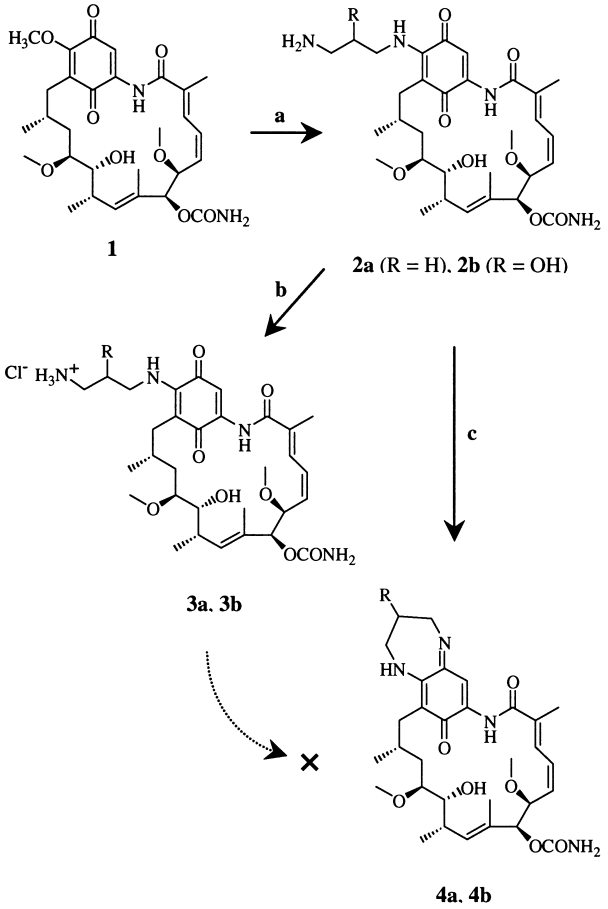

Figure 1. Synthesis of AP-GDM and AP(OH)-GDM and the protection of their primary amino group to avoid intramolecular imine formation. **1**, GDM; **2a**, AP-GDM; **2b**, AP(OH)-GDM; **3a**, AP-GDM·HCl; **3b**, AP(OH)-GDM·HCl; **4a**, 17,18-diazepino-17-demethoxygeldanamycin; **4b**, 17,18-(6-hydroxydiazepino)-17-demethoxygeldanamycin; (a) 1,3-diaminopropane; 1,3-diamino-2-hydroxypropane in chloroform; (b) HCl in NaCl-saturated water; (c) on silica gel, or probably spontaneously in alkaline aqueous media.

AP(OH)-GDM·HCl was synthesized by the same procedure as AP-GDM·HCl. The yields of two batches were 76 and 92%. Although some racemization was suggested, the synthesis of AP(OH)-GDM was validated by two-dimensional NMR analysis (Table 1), mass spectrometry, and elemental analysis.[15] This compound also changed structure (cyclization) on silica gel and in alkaline aqueous media as AP-GDM, validating the need for the described synthetic procedures for substituents capable of forming seven-membered cyclics.

Antiproliferative activity was evaluated using the human ovarian carcinoma cell line, A2780.[12] The cells growing exponentially in 96-well microtiter plates

Table 1. Two-dimensional NMR analysis of AP(OH)-GDM·HCl

	Proton	^{1}H	^{13}C
GDM moiety	2-CH_3	1.91, s, 3H	13
	H3	7.02, br s, 1H	128
	H4	6.58, t (J = 11 Hz), 1H	126
	H5	5.77, br s, 1H	138
	H6	4.39, m, 1H	81
	6, 12-OCH_3	3.19, d (J = 6 Hz), 3H	56
		3.21, d (J = 2 Hz), 3H	56
	H7[a]	4.96, s, 0.6H	79.9
		5.00, s, 0.4H	79.6
	7-$OCONH_2$	6.20–6.80, br, 2H	—
	8-CH_3	1.62, s, 3H	13
	H9	5.50, br s, 1H	131
	H10	2.55, m, 1H	32
	10-CH_3	0.94, d (J = 6.5 Hz), 3H	23
	H11[a]	3.26, m, 0.5H	72.2
		3.33, m, 0.5H	72.2
	11-OH	6.89, br s, 1H	—
	H12	3.19, br s, 1H	81
	H13	1.49, br s, 2H	32
	H14	1.85 br, 1H	28
	14-CH_3	0.80, dd (J = 7, 12 Hz), 3H	13
	H15	2.29, m, 1H	32
		2.54, m, 1H	32
	H19	6.93, br s, 1H	108
	22-NH	9.32, br s, 1H	—
DAP(OH) moiety	1′-NH	(not detected)	—
	1′-CH_2	2.74, m, 1H	42
		2.92, m, 1H	42
	2′-CH	3.93, m, 1H	66
	2′-OH	(not detected)	
	3′-CH_2	3.52, br s, 2H	48
	3′-NH_3Cl	8.05, d (5.5 Hz), 3H	—

[a]The carbon atoms attached to these hydrogen atoms may have racemized.

Table 2. Antiproliferative activity of GDM, AP-GDM, and AP(OH)-GDM towards A2780 cells

Compounds	IC_{50} dose (nM)[a]
GDM	3.4±4.3
AP-GDM	136±28
AP(OH)-GDM	234±39

[a]Data indicate mean±SE from $n=8$.

(10,000 cells/well) were incubated with drug solutions in RPMI-1640 supplemented with 10% bovine fetal serum and 10 μg/mL insulin at 37 °C in humidified atmosphere with 5% CO_2. The viability of the cells was inferred by the MTT assay. Table 2 shows the IC_{50} doses of intact GDM and its derivatives. The activity of AP-GDM was lower than that of intact GDM by 1–2 orders of magnitude. This degree of reduction was consistent with a previous report where a different evaluation system (cell line, evaluation method for cell viability, and the form of AP-GDM) was applied.[10] The activity of AP(OH)-GDM was 2 times lower than the activity of AP-GDM. However, total efficacy of the DDS is affected not only by the intrinsic activity of the drug molecule but also by the physical properties of the DDS, such as water solubility, surface hydrophobicity and drug release rate.[16] For example, in the DDS based on water soluble polymers, the 2′-hydroxy group of AP(OH)-GDM may increase the hydrophilicity of the polymer–drug conjugates. It is expected that the increased hydrophilicity allows higher drug content in the DDS, suppressing the formation of intra- or intermolecular aggregates[17,18] that could decrease therapeutic efficacy of the conjugate. In addition, different 17-substituents may result in different drug release rates. The synthesis of AP(OH)-GDM has provided another GDM derivative that is expected to have a different compatibility with DDS than AP-GDM.

In conclusion, an improved procedure to synthesize AP-GDM·HCl was developed. It substantially increased the yield of AP-GDM·HCl synthesis and could be applied to the synthesis of a novel GDM derivative with increased hydrophilicity, AP(OH)-GDM·HCl. Both GDM derivatives possessed antiproliferative activities toward A2780 human ovarian carcinoma cells.

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15. Typical synthetic procedure for AP-GDM·HCl: GDM (140 mg, 250 μmol, kindly supplied by the NCI) was dissolved in chloroform (20 mL) at 40 °C, and the solution was cooled to 25 °C. 1,3-Diaminopropane (500 mg, 6.7 mmol) was added to the solution. The mixture was stirred at 25 °C for 2 h. NaCl-saturated water (50 mL) was added to the mixture. Neutralization with 6 N HCl resulted in the precipitation of AP-GDM·HCl from the mixture. The precipitate was collected by filtration, washed with chloroform, and dissolved in the mixture of chloroform and 5% $NaHCO_3$ aqueous solution (100 mL each). The organic layer containing AP-GDM was collected, and the product was further extracted with chloroform (50 mL×3). The organic layers were combined, washed with NaCl-saturated water (pH adjusted to 8 with $NaHCO_3$), and dried over Na_2SO_4. After Na_2SO_4 was filtered off, the organic layer was evaporated at 30 °C to dryness. The residue was dissolved in CH_3OH (2 mL), and 6 N HCl (84 μL, 500 μmol of HCl) was added. AP-GDM·HCl was precipitated with ether (100 mL). The precipitate was collected by filtration and dried under reduced pressure. Mass spectroscopy was performed on a mass spectrometer Voyager-DE (STR Biospectrometry Workstation, PerSeptive Biosystem, Inc., Framingham, MA, USA). Elemental analysis was performed by Atlantic Microlab, Inc., Norcross, GA. Two-dimensional NMR was performed on a spectrometer Varian Unity 500 MHz. DMSO-d_6 was used as a solvent in NMR measurements. R_f 0.33 for AP-GDM·HCl, 0.53 for AP(OH)-GDM·HCl on silica gel and AcOEt/MeOH (2:1); *m/e* 604.3 for AP-GDM ($M^+ + 1$ of free base), 619.0 for AP(OH)-GDM ($M^+ + 1$ of free base). Anal. calcd for AP-GDM·HCl·$2H_2O$ ($C_{31}H_{47}O_8N_4$ Cl·$2H_2O$; C, 55.14; H, 7.61; N, 8.30; found: C, 55.25; H, 7.51; N, 8.25; calcd for AP(OH)-GDM·HCl·$2H_2O$ ($C_{31}H_{47}O_9N_4Cl$·$2H_2O$; C, 53.87; H, 7.44; N, 8.11; found: C, 53.70; H, 7.53; N, 8.06. Synthetic data for AP-GDM·HCl were cited from ref 12.
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