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New tricyclic geldanamycin analogues from an engineered strain of *Streptomyces hygroscopicus* JCM4427

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ABSTRACT

Two tricyclic geldanamycin analogues, DHQ5 (**1**) and DHQ6 (**2**), were produced by a combinatorial mutant (AC15) contained a site-directed mutagenesis on the geldanamycin polyketide synthase (PKS) gene with inactivation of the post-PKS tailoring genes (*gel7*) of *Streptomyces hygroscopicus* JCM4427. The structural diversity of tricyclic geldanamycin analogues is due to the formation of unusual additional rings, which are formed by alkylation of the C-21 position by C-12 in DHQ5 (**1**) and by electrophilic addition of the C-15 hydroxyl group to the double bond (C-8–C-9), which leads to the migration of the double bond (to C-7–C-8) and the elimination of a carbamoyloxy group in DHQ6 (**2**).

Geldanamycin (GM), an ansamycin anticancer agent produced by *Streptomyces hygroscopicus*, binds to the ATP-binding site of heat shock protein 90 (HSP90) and inhibits its ATP-dependent chaperone activities, which are critical for its interactions with numerous oncogenic proteins required for signal transduction and transcription during tumorigenesis in cancer cells.[1,2] Therefore, GM and its analogues are considered as potential anticancer chemotherapeutic agents.[3,4] However, due to the occurrence of unwanted side effects of the current analogues during preclinical and clinical trials,[5] new GM analogues with improved pharmacological profiles are needed.

GM is produced in several *Streptomyces* strains by genes encoding a modular polyketide synthase (PKS) and several tailoring enzymes.[6] A clear understanding of GM biosynthesis would facilitate the generation of structurally diverse GM analogues with optimal biological activities. In our previous studies, we identified the GM biosynthetic gene cluster in *S. hygroscopicus* subsp. *duamyceticus* JCM4427 and characterized the GM biosynthetic pathway.[7–9] Moreover, during these studies, novel GM analogues were prepared from several genetically engineered strains, and subsequently their biological potentials were evaluated.[10,11] Recently, we described the designed biosynthesis of C-15 hydroxylated non-quinone GM analogues by site-directed mutagenesis of the GM PKS gene, and by utilizing a combination of post-PKS tailoring genes. Of these GM analogues, DHQ3, a 15-hydroxyl-17-demethoxy non-quinone analogue from a *gel7* gene inactivation mutant (AC15) of *S. hyg*-DHQ, was found to inhibit HSP90 ATPase activity more than GM.[10] In this Letter, we report the isolation and structure elucidation of new tricyclic GM analogues, DHQ5 (**1**) and DHQ6 (**2**) (Fig. 1), which were produced as minor metabolites from the *gel7* gene inactivation mutant (AC15) of *S. hyg*-DHQ strain.

DHQ5 (**1**) was obtained as a colorless powder and its molecular formula was established as $C_{29}H_{41}N_3O_9$ based on the HRFABMS (positive mode; $[M+Na]^+$ m/z 598.2742, calcd 598.2741), which requires 11 degrees of unsaturation. In its ^1H NMR spectrum, two characteristic aromatic signals at δ_H 7.92 (1H, d, J = 2.8 Hz, H-19) and 6.51 (1H, d, J = 2.4 Hz, H-17); two olefinic methine signals at δ_H 6.19 (1H, br t, J = 7.5 Hz, H-3) and 5.54 (1H, d, J = 9.6 Hz, H-9); four oxygenated methine signals at δ_H 5.21 (1H, d, J = 6.4 Hz, H-11), 5.11 (1H, d, J = 8.8 Hz, H-7), 4.36 (1H, d, J = 2.0 Hz, H-15), and 3.57 (1H, m, H-6); two methoxyl signals at δ_H 3.41 (3H, s, 6-OCH_3) and 3.21 (3H, s, 12-OCH_3); and four methyl signals at δ_H 1.92 (3H, s, H-22), 1.71 (3H, s, H-23), 1.17 (3H, d, J = 6.4 Hz, H-25), and 0.89 (3H, d, J = 7.2 Hz, H-24) were observed. The ^{13}C and DEPT NMR spectra of **1** showed 29 carbon signals including six methyls, three methylenes, ten methines, and ten quaternary carbons, of which the signals of an amide carbonyl carbon (δ_C 169.7), five oxygen-bearing sp^3 hybridized carbons (δ_C 83.7, 82.1, 81.9, 78.1, and 73.3), and two methoxyl groups (δ_C 58.2 and 51.7) were typical. Based on these observations and a comparison of its spectroscopic data with those of DHQ3 previously isolated from the

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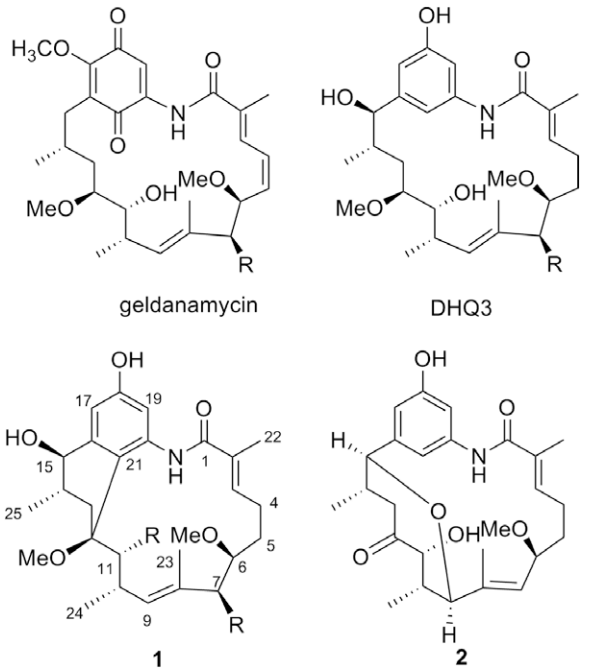

Figure 1. Structures of geldanamycin, DHQ3, and compounds **1** and **2** (R = $OCONH_2$).

same engineered strain (AC15),[10] compound **1** was suggested to be a C-15 hydroxylated non-quinone GM.

The identification of the tricyclic skeleton of compound **1** was one of the most challenging parts of the structure elucidation. Based on previous observations, one amide, two carbamoyls, two double bonds, one benzene ring, and an ansamacrolide ring accounted for ten of the total 11 degrees of unsaturation, and hence, the presence of an additional ring was inferred from the extra degree of unsaturation. The absence of the oxygenated methine and aromatic proton signals for C-12 and C-21 of DHQ5 (**1**) in its ^{1}H NMR spectrum, and HMBCs of H-11 (δ_H 5.21) and H-13 (δ_H 1.89) to C-21 (δ_C 114.3) suggested the formation of an additional ring (Fig. 2). Connectivity between C-12 and C-21 was further verified by HMBCs. The methine proton signal at δ_H 4.36 (H-15) was correlated with aromatic carbon signals at δ_C 145.9 (C-16), 113.4 (C-17), and 114.3 (C-21) and the amide proton signal at δ_H 10.23 showed the correlations with aromatic carbon signals at δ_C 109.1 (C-19), 140.6 (C-20), and 114.3 (C-21) in the HMBC NMR experiment (Fig. 2). These correlations indicate that C-12 was connected to C-21 and not to C-17.

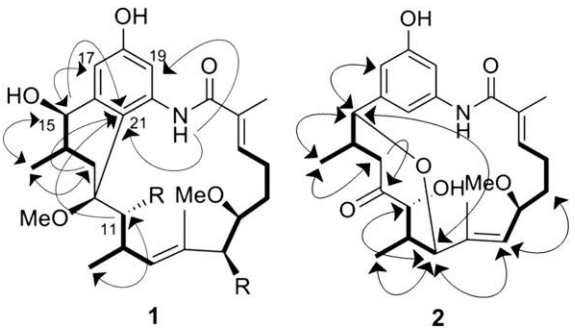

Figure 2. Key ^{1}H–^{1}H COSY (bold) and HMBCs (→) of **1** and **2**.

The positions of the functional groups were determined unambiguously as C-7 and C-11 (two carbamoyls), C-15 and C-18 (two hydroxyls), and C-6 and C-12 (two methoxyls) with 2D NMR techniques. The stereochemistry of **1** was inferred from biosynthetic considerations, which indicated that the configuration of the ansamacrolide ring is the same as that of GM, and consideration of ROESY correlations of H-15 to H-14, H-17, and H-25, which suggested a pseudo-equatorial H-15 (Fig. 3). These correlations were supported by calculated interatomic distances of less than 2.5 Å (energy-minimized structure by MMFF94 force field molecular modeling, CS ChemBio3D, V 11.0). Accordingly, the structure of the new tricyclic GM analogue, DHQ5 (**1**), was elucidated (Table 1).[12]

The mass spectrometric analysis of DHQ6 (**2**) provided a molecular formula of $C_{26}H_{35}NO_6$ (positive HRFABMS *m*/*z* 480.2360 $[M+Na]^+$; calcd 480.2362), with 10 degrees of unsaturation. The ^{1}H NMR spectrum of **2** exhibited three aromatic signals at δ_H 6.50 (1H, br t, H-17), 6.38 (1H, t, J = 2.0 Hz, H-19), and 6.22 (1H, br t, H-21); two olefinic signals at δ_H 5.37 (1H, overlap, H-7) and 5.36 (1H, overlap, H-3); four oxygen-bearing methine signals at δ_H 4.03 (1H, d, J = 9.2 Hz, H-11), 3.99 (1H, m, H-6), 3.86 (1H, d, J = 10.4 Hz, H-9), and 3.82 (1H, d, J = 10.0 Hz, H-15); one methoxyl signal at δ_H 3.21 (3H, s, 6-OCH_3); and four methyl signals at δ_H 1.78 (3H, s, H-22), 1.41 (3H, s, H-23), 0.95 (3H, d, J = 6.8 Hz, H-24), and 0.79 (3H, d, J = 6.8 Hz, H-25). The ^{13}C NMR and DEPT spectra of **2** displayed 26 carbon signals including a ketone (δ_C 215.0), an amide (δ_C 176.0), four oxygen-bearing sp^3 hybridized methines (δ_C 93.4, 92.1, 82.3, and 77.9), a methoxyl (δ_C 56.5), three sp^3 hybridized methylenes (δ_C 48.5, 34.3, and 22.5), and four methyls (δ_C 17.2, 14.9, 14.5, and 11.3). Therefore, it was inferred that compound **2** also has a C-15 hydroxylated non-quinone GM skeleton without a carbamoyl group, accounting for nine degrees of unsaturation,

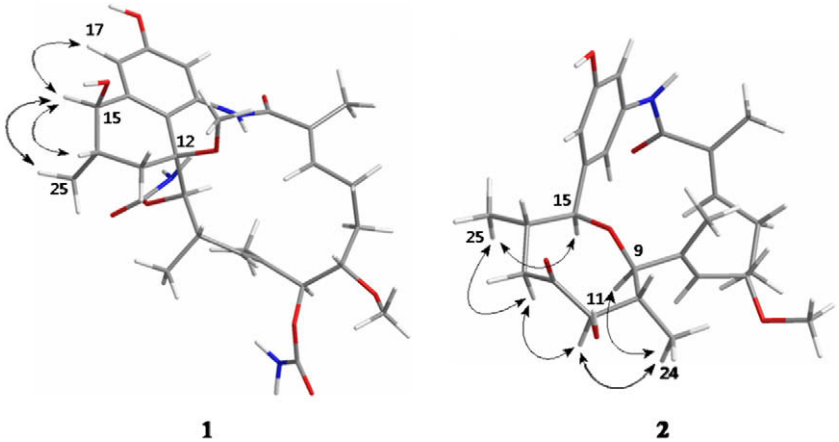

Figure 3. Energy minimized structures and key ROESY correlations of **1** and **2**.

Table 1
NMR data of DHQ5 (**1**) and DHQ6 (**2**) in CD_3OD

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		169.7 s[a]		176.0 s[a]
2		134.6 s		130.4 s
3	6.19 br t (7.5)	137.2 d	5.36[b]	139.0 d
4	2.51 m, 2.31 m	21.6 t	2.18[b], 1.97 m	22.5 t
5	2.26 m, 1.40 m	27.8 t	1.83 m, 1.14 m	34.3 t
6	3.57 m	81.9 d	3.99 m	77.9 d
7	5.11 d (8.8)	82.1 d	5.37[b]	131.7 d
8		130.8 s		138.3 s
9	5.54 d (9.6)	140.5 d	3.86 d (10.4)	93.4 d
10	3.12 m	33.9 d	2.12 m	49.1 d
11	5.21 d (6.4)	78.1 d	4.03 d (9.2)	82.3 d
12		83.7 s		215.0 s
13	2.43 d (14.4)	32.7 t	2.71 t (12.8)	48.5 t
	1.89 dd (14.8, 4.4)		2.22 dd (12.8, 2.8)	
14	2.00 m	34.0 d	2.51 m	47.5 d
15	4.36 d (2.0)	73.3 d	3.82 d (10.0)	92.1 d
16		145.9 s		144.4 s
17	6.51 d (2.4)	113.4 d	6.50 br t	110.4 d
18		158.7 s		159.7 s
19	7.92 d (2.8)	109.1 d	6.38 t (2.0)	111.0 d
20		140.6 s		141.1 s
21		114.3 s	6.22 br t	117.3 d
22	1.92 s	13.0 q	1.78 s	14.5 q
23	1.71 s	11.9 q	1.41 s	11.3 q
24	0.89 d (7.2)	17.2 q	0.95 d (6.8)	14.9 q
25	1.17 d (6.4)	17.8 q	0.79 d (6.8)	17.2 q
6-OCH_3	3.41 s	58.2 q	3.21 s	56.5 q
12-OCH_3	3.21 s	51.7 q		
7-$OCONH_2$		159.6 s		
11-$OCONH_2$		159.1 s		
NH	10.23 s			

[a] Carbon multiplicity.
[b] Multiplicity patterns were unclear due to signal overlapping.

and also suggesting the presence of an additional ring from the extra degree of unsaturation.

The gross structure of **2** was established based on 2D NMR data. The HMBCs of H-5 (δ_H 1.83) and H-6 (δ_H 3.99) to C-7 (δ_C 131.7), and H-7 (δ_H 5.37) to C-5 (δ_C 34.3), C-9 (δ_C 93.4), and C-23 (δ_C 11.3), and the assignment of the H-3–H-7 proton spin system based on ^1H–^1H COSY correlations positioned the double bond at C-7 (Fig. 2) (this is usually located at C-8 in normal GM analogues). Also, the position of the ketone group was determined as C-12 from HMBCs between H-13 (δ_H 2.71 and 2.22) and C-12 (δ_C 215.0). The presence of an oxide bridge between C-9 and C-15 was confirmed by the downfield shifted signals of C-9 (δ_C 93.4) and C-15 (δ_C 92.1), and H-9/C-15 and H-15/C-9 HMBCs. The stereochemistry of **2** was also inferred from biosynthetic considerations as well as consideration of ROESY correlations (H-9/H-24, H-24/H-11, H-11/H-13α, H-13α/H-25, and H-25/H-15) (Fig. 3), and this was found to be in good agreement with the energy-minimized structure of **2** determined by MMFF94 molecular modeling. Therefore, the structure of DHQ6 (**2**) was assigned as shown.[13]

Previously two tricyclic GM analogues, DHQ1 and DHQ2 (KOSN-1633), were identified from *S. hyg*-DHQ mutant and from the microbial bioconversion.[10,14] In these compounds, the additional ring resulted from dehydrative cyclization involving two hydroxyl groups at C-11 and C-15. However, the structures of DHQ5 (**1**) and DHQ6 (**2**) imply that the additional rings of **1** and **2** are unusually formed by the alkylation of the C-21 position by C-12 and by electrophilic addition to the double bond (C-8–C-9) by the C-15 hydroxyl group, which leads eventually to double bond (C-7–C-8) migration and to the elimination of the carbamoyloxy group, respectively. DHQ5 (**1**) contains two carbamoyl groups at C-7 and C-11, and this unusual bis-carbamoylation on a linear polyketide was also observed during our previous studies.[11] Taken together, the carbamoyl transferase (Gel8), a post-PKS modification enzyme, can recognize two hydroxyl groups at the C-7 and C-11 positions. DHQ5 (**1**) and DHQ6 (**2**) were evaluated for their potential to inhibit human breast cancer cell line (SKBr3) and HSP90 ATPase activity according to established protocols,[10,15] and did not exhibit any inhibitory activity in both assay systems (IC_{50} values, >20 μg/mL).

Acknowledgments

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Supplementary data

Experimental details, and 1D and 2D NMR spectra of DHQ5 (**1**) and DHQ6 (**2**). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.11.018.

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12. DHQ5 (**1**). Amorphous powder; mp 216 °C; $[\alpha]_D^{25}$ +61.6 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε): 225 (4.06), 275 (3.67) nm; IR (ATR) ν_{max} cm^{-1}: 3340, 2929, 1677, 1599; ESIMS *m/z* 598.6 $[M+Na]^+$, 1173.7 $[2M+Na]^+$, HRFABMS (positive) *m/z* 598.2742 $[M+Na]^+$ (calcd. for $C_{29}H_{41}N_3O_9Na$, 598.2741); ^1H and ^{13}C NMR data, see Table 1.
13. DHQ6 (**2**). Amorphous powder; mp 205 °C; $[\alpha]_D^{25}$ −25.2 (*c* 0.67, MeOH); UV (MeOH) λ_{max} (log ε): 225 (4.11), 285 (3.61) nm; IR (NaCl) ν_{max} cm^{-1}: 3279, 2927, 1661, 1597; ESIMS *m/z* 480.6 $[M+Na]^+$, 937.8 $[2M+Na]^+$, 456.6 $[M-H]^-$, 913.9 $[2M-H]^-$; HRFABMS (positive) *m/z* 480.2360 $[M+Na]^+$ (calcd for $C_{26}H_{35}NO_6Na$, 480.2362); ^1H and ^{13}C NMR data, see Table 1.
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