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Supporting Information

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DOI: 10.1002/cbic.200800763

Supporting Information

for

Rational Biosynthetic Engineering for Optimization of Geldanamycin Analogues

Woncheol Kim, Dongho Lee, Seong Su Hong, Zhu Na, Jin Chul Shin, Su Heun Roh, Cheng-Zhu Wu, Oksik Choi, Kyeong Lee, Yue-Mao Shen, Sang-Gi Paik, Jung Joon Lee, and Young-Soo Hong*

Structure Elucidation

DHQ1

^{1}H NMR (CD_3OD, 400 MHz) δ 0.85 (3H, d, *J* = 6.4 Hz, H-25), 0.89 (3H, d, *J* = 7.2 Hz, H-24), 1.69 (2H, m, H-5), 1.79 (3H, s, H-23), 1.92 (3H, s, H-22), 2.40 (2H, m, H-4), 2.76 (1H, m, H-10), 3.37 (3H, s, 6-OCH_3), 3.41 (1H, brt, H-12), 3.52 (3H, s, 12-OCH_3), 3.60 (1H, t, *J* = 5.2 and 5.6 Hz, H-6), 4.10 (1H, d, *J* = 9.2 Hz, H-11), 4.52 (1H, d, *J* = 10.8 Hz, H-15), 5.05 (1H, brs, H-7), 5.42 (1H, d, *J* = 9.6 Hz, H-9), 6.42 (1H, t, *J* = 7.2 and 7.6 Hz, H-3), 7.21 (1H, s, H-19), 9.01 (1H, s, NH). ^{13}C NMR (CD_3OD, 100 MHz) δ 12.06 (C-22), 12.46 (C-23 and 24), 17.47 (C-25), 23.86 (C-4), 27.45 (C-14), 30.54 (C-5), 32.58 (C-10), 35.14 (C-13), 56.24 (6-OCH_3), 59.75 (12-OCH_3), 74.68 (C-11), 75.67 (C-15), 76.06 (C-12), 80.04 (C-7), 84.80 (C-6), 106.15 (C-19), 116.57 (C-16), 131.99 (C-9), 132.16 (C-8), 134.33 (C-2), 137.62 (C-3), 141.31 (C-20), 153.12 (C-17), 156.41 ($CONH_2$), 168.53 (C-1), 181.74 (C-18), 182.50 (C-21). ESI-MS *m/z* 569 [*M* + Na]$^+$, 545 [*M* – H]$^-$. HRFABMS *m/z* 569.2473, calcd for $C_{28}H_{38}N_2O_9Na$ ([*M* + Na]$^+$) 569.2475.

HMBC correlations : H-3/C-1, C-2, C-4, C-5, C-22, H-4/C-2, C-3, C-5, C-6, H-6/OCH_3, H-7/C-9, C-23, $OCONH_2$, H-9/C-7, C-10, C-11, C-23, H-10/C-9, C-24, H-11/C-9, C-10, C-15, C-24, H-12/OCH_3, H-13/C-11, C-12, H-15/C-11, C-13, C-14, C-16, C-17, C-21, C-25, H-19/C-17, C-20, C-21, H-22/C-1, C-2, C-3, H-23/C-7, C-9, H-24/C-9, C-10, C-11, H-25/C-13, C-14, C-15, NH/C-1, C-19, C-21.

DHQ2 (Hu et al. *J. Antibiotics* **2004**, *57*, 421-428.[1])

^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, d, *J* = 6.8 Hz, H-25), 0.90 (3H, d, *J* = 7.6 Hz, H-24), 1.58 (1H, m, H-13), 1.78 (3H, s, H-23), 1.95 (1H, m, H-13), 2.02 (3H, s, H-22), 2.60 (1H, m, H-14), 2.74 (1H, m, H-10), 3.39 (3H, s, 12-OCH_3), 3.41 (3H, s, 6-OCH_3), 3.45 (1H, m, H-12), 4.17 (1H, d, *J* = 9.6 Hz, H-11), 4.41 (1H, d, *J* = 7.6 Hz, H-6), 4.53 (1H, d, *J*=11.2 Hz, H-15), 5.07 (1H, s, H-7), 5.50 (1H, d, *J* = 9.2 Hz, H-9), 5.91 (1H, dd, *J* = 11.1, 7.4 Hz, H-5), 6.51 (1H, t, *J* = 11.0 Hz, H-4), 7.06 (1H, d, *J* = 11.6 Hz, H-3), 7.29 (1H, s, H-19), 9.25 (1H, s, NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.89 (C-22), 12.24 (C-24), 12.71 (C-23), 17.46 (C-25), 27.29 (C-14), 32.35 (C-10), 35.38 (C-13), 56.27 (12-OCH_3), 58.09 (6-OCH_3), 74.17 (C-11), 75.71 (C-15), 75.92 (C-12), 82.07 (C-7), 83.74 (C-6), 106.73 (C-19), 116.58 (C-16), 125.61 (C-4), 129.55 (C-3), 132.25 (C-8), 132.53 (C-9), 133.63 (C-2), 139.41 (C-5), 141.37 (C-20), 153.13 (C-17), 155.78 (7-$OCONH_2$), 167.34 (C-1), 182.21 (C-21), 182.56 (C-18).). ESI-MS *m/z* 567 $[M + Na]^+$, 543 $[M - H]^-$. HRFABMS *m/z* 567.2319, calcd for C28H36N2O9Na ($[M + Na]^+$) 567.2319

HMBC correlations : H-3/C-1, C-5, C-22, H-4/ C-6, H-5/C-3, C-6, H-6/OCH_3, H-7/C-9, C-23, $OCONH_2$, H-9/C-7, C-10, C-11, C-23, H-11/C-9, C-10, C-15, C-24, H-12/C-10, C-11, C-14, OCH_3, H-13/C-12, C-14, H-15/C-11, C-13, C-14, C-17, C-21, C-25, H-19/C-17, C-18, C-20, H-22/C-1, C-2, C-3, H-23/C-7, C-8, C-9, H-24/C-9, C-10, C-11, H-25/C-13, C-14, C-15, NH/C-1, C-19, C-21, 17-OH/C-16, C-17, C-18.

DHQ3

^{1}H NMR (CD_3OD, 400 MHz) δ 0.65 (3H, d, *J* = 7.2 Hz, H-25), 0.75 (1H, brt, *J* = 10.8 Hz, H-13), 1.02 (3H, d, *J* = 6.8 Hz, H-24), 1.11 (2H, m, H-5), 1.77 (1H, dt, *J* = 3.6 and 10.8 Hz, H-13), 1.37 (3H, s, H-23), 1.86 (3H, s, H-22), 2.04 (1H, m, H-4), 2.34 (1H, m, H-10), 2.72 (overlap, H-4), 3.07 (1H, dt, *J* = 4.0 and 11.2 Hz, H-12), 3.22 (1H, t, *J* = 8.4 Hz, H-6), 3.35 (3H, s, 12-OCH_3), 3.42 (3H, s, 6-OCH_3), 3.64 (1H, dd, *J* = 2.8 and 9.2 Hz, H-11), 4.47 (1H, d, *J* = 4.0 Hz, H-15), 4.87 (overlap, H-7), 5.16 (1H, d, *J* = 9.6 Hz, H-9), 5.58 (1H, brd, *J* = 7.2 Hz, H-3), 6.37 (1H, s, H-17), 6.49 (1H, s, H-19). ^{13}C NMR (CD_3OD, 100 MHz) δ 12.14 (C-23), 12.25 (C-25), 14.23 (C-22), 18.43 (C-24), 23.88 (C-4), 31.19 (C-5), 31.61 (C-13), 36.22 (C-10 and 14), 57.12 (12-OCH_3), 59.89 (6-OCH_3), 74.12 (C-11), 79.25 (C-15), 80.30 (C-12), 80.75 (C-6), 83.97 (C-7), 109.90 (C-19), 113.41 (C-17), 116.28 (C-21), 131.68 (C-8), 131.83 (C-2), 134.28 (C-9), 135.56 (C-3), 141.45 (C-20), 144.85 (C-16), 158.52 (C-18), 159.14 ($CONH_2$), 176.01 (C-1). ESI-MS *m/z* 557 $[M + Na]^+$, 533 $[M - H]^-$. ESI-TOF-MS *m/z* 552.3279, calcd for $C_{28}H_{46}N_3O_8$ ($[M + NH_4]^+$) 552.3304.

HMBC correlations : H-3/C-1, C-22, H-4/C-2, C-3, C-5, C-6, H-5/C-3, C-4, C-6, C-7, H-6/C-4, C-5, C-7, OCH_3, H-7/C-5, C-6, C-8, C-9, C-23, $OCONH_2$, H-9/C-7, C-10, C-11, C-23, C-24, H-10/C-9, C-11, H-11/C-10, C-12, C-13, C-24, H-13/C-11, C-12, C-25, H-15/ C-13, C-14, C-

16, C-17, C-21, C-25, H-17/C-15, C-19, H-19/C-18, H-21/C-15, C-16, C-17, C-19, C-20, H-22/C-1, C-2, C-3, H-23/C-7, C-8, C-9, H-24/C-9, C-10, C-11, H-25/C-13, C-14, C-15, OCH_3/C-12.

DHQ4

^{1}H NMR (pyridine-d_5, 400 MHz) δ 1.20 (3H, d, *J* = 6.8 Hz, H-25), 1.25 (3H, d, *J* = 6.4 Hz, H-24), 1.49 (2H, m, H-5), 1.89 (3H, s, H-23), 2.11 (3H, s, H-22), 2.32 (1H, m, H-4), 2.40 (overlap, H-4), 2.84 (1H, m, H-10), 3.45 (3H, s, 12-OCH_3), 3.49 (1H, m, H-12), 3.55 (1H, m, H-6), 3.61 (3H, s, 6-OCH_3), 3.99 (1H, dd, *J* = 4.0 and 7.6 Hz, H-11), 4.29 (1H, d, *J* = 7.6 Hz, H-7), 4.96 (1H, d, *J* = 4.4 Hz, H-15) 5.46 (1H, d, *J* = 7.2 Hz, H-9), 6.22 (1H, brs, H-3), 7.04 (1H, s, H-17), 7.45 (1H, brs, H-19), 10.64 (1H, s, NH). ^{13}C NMR (CD_3OD, 100 MHz) δ 12.56 (C-23), 14.07 (C-25), 14.58 (C-22), 18.13 (C-24), 24.11 (C-4), 31.18 (C-5), 31.93 (C-13), 35.46 (C-10), 36.64 (C-14), 57.39 (12-OCH_3), 59.83 (6-OCH_3), 74.37 (C-11), 78.52 (C-15), 80.98 (C-12), 82.41 (C-7), 82.62 (C-6), 108.57 (C-19), 112.56 (C-17), 114.88 (C-21), 131.60 (C-9), 132.61 (C-2), 135.41 (C-3), 136.14 (C-8), 142.30 (C-20), 146.52 (C-16), 159.63 (C-18), 173.41 (C-1). ESI-MS *m/z* 514 $[M + Na]^+$, 490 $[M - H]^-$. HRFABMS *m/z* 514.2786, calcd for $C_{27}H_{41}NO_7Na$ ($[M + Na]^+$) 514.2781.

HMBC correlations : H-3/C-1, C-22, H-4/C-2, C-3, C-5, C-6, H-5/C-3, C-4, C-6, H-6/C-4, C-5, C-7, OCH_3, H-7/C-5, C-6, C-8, C-9, C-23, H-11/C-10, C-12, C-13, C-24, H-12/OCH3, H-15/ C-13, C-14, C-16, C-17, C-21, C-25, H-17/C-15, C-18, C-21, H-19/C-21, H-21/C-15, C-17, C-19, H-22/C-2, C-3, H-23/C-7, C-8, C-9, H-24/C-9, C-10, C-11, H-25/C-13, C-14, C-15, NH/C-1, C-19, C-21.

References

[1] Z. Hu, Y. Liu, Z. Q. Tian, W. Ma, C. M. Starks, R. Regentin, P. Licari, D. C. Myles, C. R. Hutchinson, *J. Antibiot.* **2004**, *57*(7), 421-428.

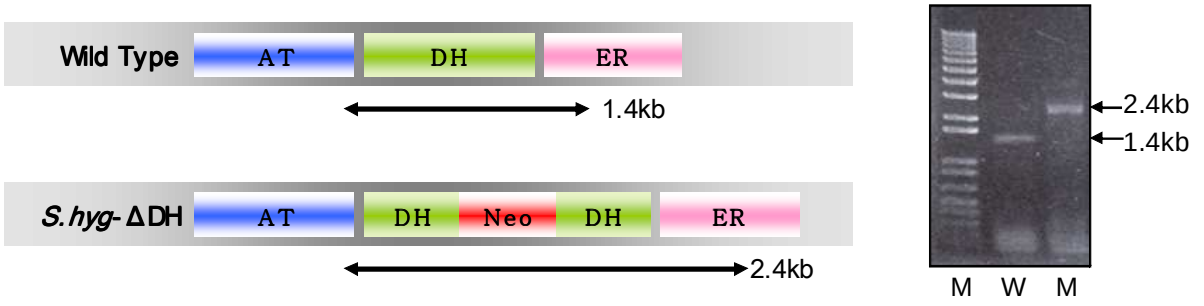

Figure S1. Confirmation of insertional gene inactivation by PCR using the total genomic DNA of each mutant as template. The relevant designed primers used to amplify the desired DNA fragments are indicated by solid arrows. Mr, 1 kb ladder; W, wild type; M, mutants. PCR primers were designed around DH domain, such as 5' of DH domain region for sense primer and 870 bp downstream of sense primer region for antisense. As result, 1.4 kb of the PCR product was detected from wild type *S. hygroscopicus* and 2.4 kb of PCR product was shown from *S. hyg*-ΔDH. This result showed insertion of *aph*II gene (1 kb) in the middle of DH domain.

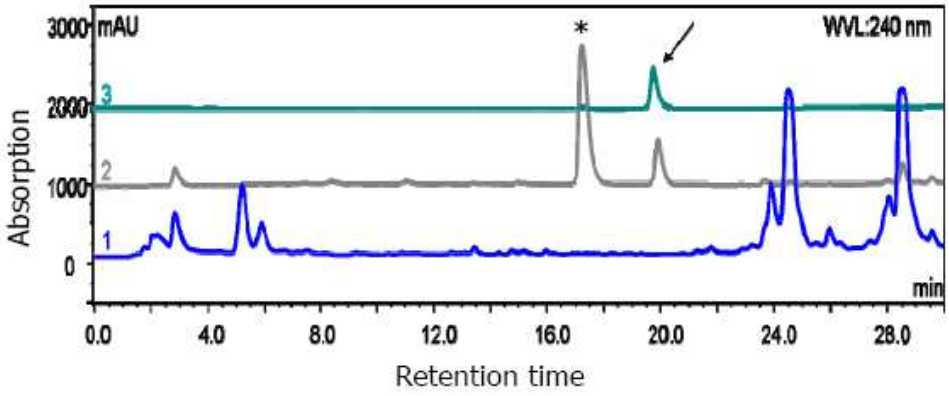

Figure S2. Comparison of HPLC analysis of ethylacetate-extracted broths of *S. hyg*-ΔDH. Crude extract of *S. hyg*-ΔDH and wild type compared with HPLC. While wild type produce geldanamycin (peak at 20min.), the mutant did not produce geldanamycin. Trace 1, crude extract of mutant *S. hyg*-ΔDH; Trace 2, crude extract of *S. hygroscopicus* JCM4427; Trace 3, geldanamycin standard. The arrow shows geldanamycin peak. [YMC J'sphere ODS-H80, 150 × 4.6mm i.d., MeOH-H_2O (0.05% acetic acid) 20:80 up to 100:0 over 25 min, 1 mL/min]. The arrows and asterisk indicate the peaks of geldanamycin and 17-demethylgeldanamycin, respectively.

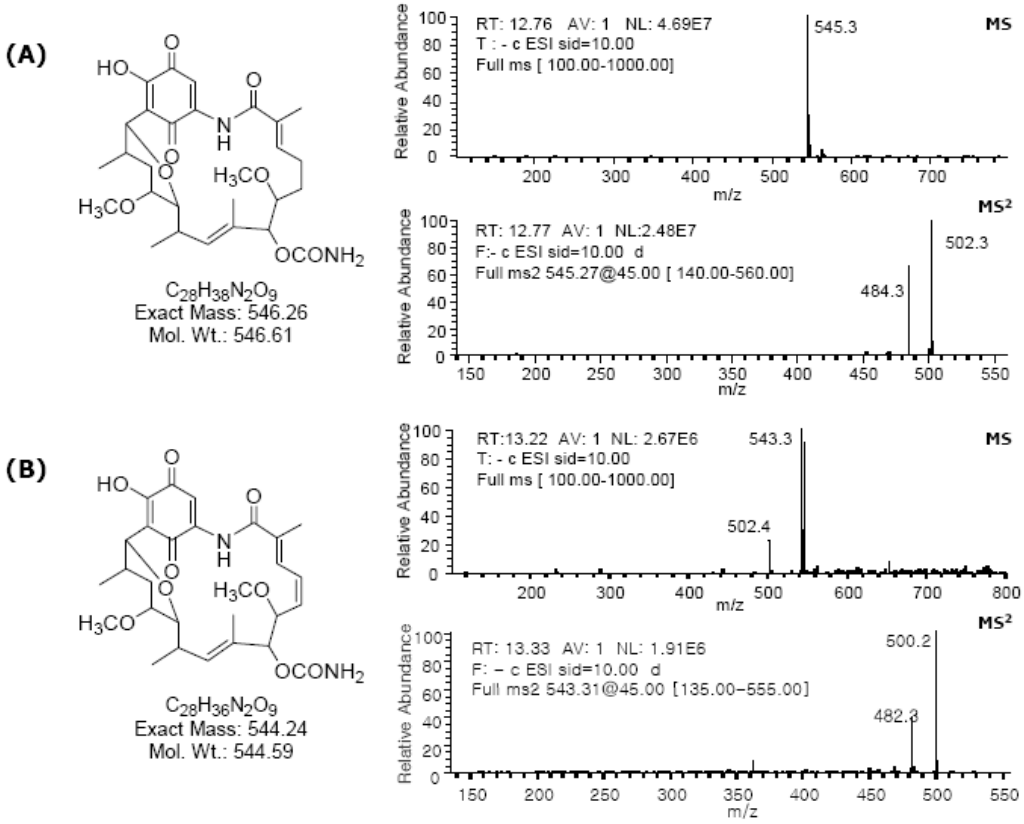

Figure S3.Structures and MS^n spectra of DHQ1 (A) and DHQ2 (B) produced by S. hyg-DHQ mutant. Peaks at m/z 545 [*M*-H]-, corresponding to DHQ1 and at m/z 543 [*M*-H]-, corresponding to DHQ2 were detected in significant amounts

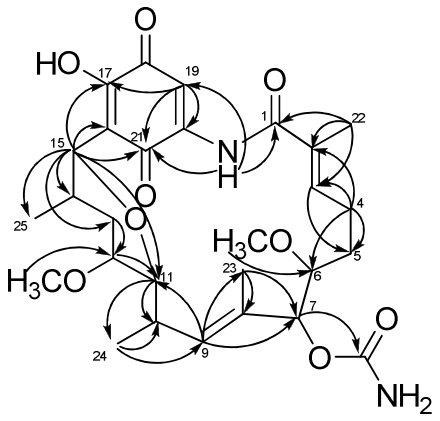

Figure S4. Key correlations of DHQ1 in HMBC (H→C) spectrum

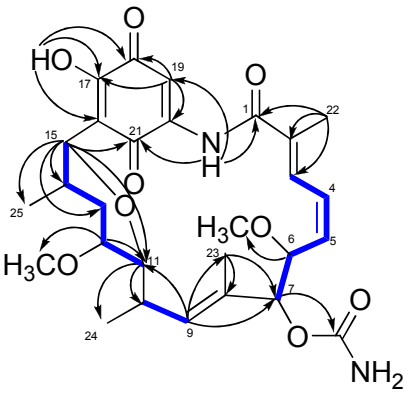

Figure S5. Key correlations of DHQ2 in HMBC (H→C) and COSY (▬) spectra

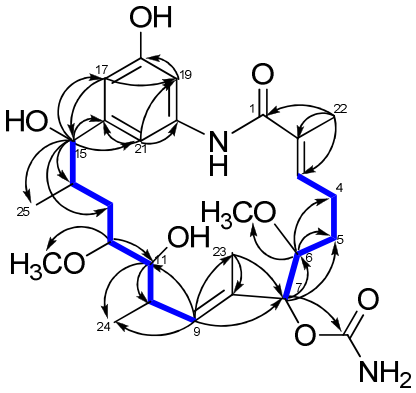

Figure S6. Key correlations of DHQ3 in HMBC (H→C) and COSY (▬) spectra

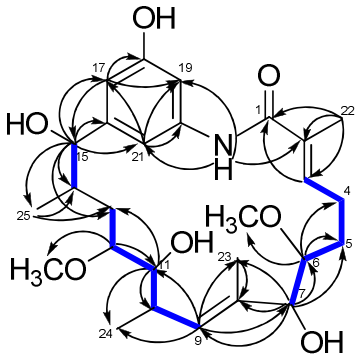

Figure S7. Key correlations of DHQ4 in HMBC (H→C) and COSY (▬) spectra