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Isolation and Characterization of Novel Geldanamycin Analogues

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New geldanamycin analogues with novel structures arising from direct microbial bioconversion and a genetically engineered geldanamycin producer were isolated and characterized. Three compounds, 15-hydroxygeldanamycin, a tricyclic geldanamycin analog (KOSN-1633), and methyl-geldanamycinate[1)], were isolated after geldanamycin was added to a growing culture of the herbimycin producing strain–*Streptomyces hygroscopicus* AM-3672. Two related compounds, 17-formyl-17-demethoxy-18-*O*,-21-*O*-dihydrogeldanamycin and 17-hydroxymethyl-17-demethoxygeldanamycin were isolated from *S. hygroscopicus* NRRL 3602/pKOS279-78, a geldanamycin-producing strain containing various genes isolated from *S. hygroscopicus* AM-3672. Compared with geldanamycin, these five new compounds exhibited reduced cytotoxicity against SKBr3 cancer cells.

The natural, closely structurally related compounds–geldanamycin, herbimycin A and reblastatin (Fig. 1), produced by different *Streptomyces* species, have been identified as Hsp90 inhibitors, and are promising drug candidates in combating several types of human cancer[2)]. These compounds are lipophilic and have low water solubility, which limits the types of formulations that can be used to administer them. One method to increase water solubility is to add ionizable or polar groups to them. Molecular modeling studies of the interaction of geldanamycin with Hsp90 suggested that substituents added at the C15 position would not interfere with binding of geldanamycin to Hsp90. Hence C15 appeared to be an ideal position for adding functional groups to improve water solubility. For this purpose, direct microbial bioconversion to introduce a hydroxyl group seemed to be practical. Microbial bioconversion, *via* hydroxylation, methylation or other modification, has been used widely to add functional groups to a wide variety of compounds[3~7)]. Based on knowledge of polyketide biosynthesis and the modular gene organization of the geldanamycin polyketide synthase (PKS) cluster[8)], we postulated that the C15-methoxy group in herbimycin A was formed by a two-step process of post-PKS modification–C15 hydroxylation and *O*-methylation. Hence, we tested whether the hydroxylase responsible for C15-hydroxylation could oxidize geldanamycin (or its derivatives) at C15 by feeding the herbimycin producing strain *Streptomyces hygroscopicus* AM-3672[9)] with geldanamycin and examining the product(s) of the fermentation. Although microbial conversion is useful for making novel analogs, it is not always feasible to produce the new compounds in large enough amount for further chemical modification. Genetic engineering may overcome this problem and generate recombinant strains that can produce the newly found analogs, if genes responsible for biosynthesis of these new analogs are characterized and identified. Successful bioconversion results encouraged us to clone the herbimycin gene cluster in the hope that a C-15 hydroxylase gene would be found clustering with polyketide synthase genes for herbimycin biosynthesis. The herbimycin biosynthesis gene cluster was cloned and sequenced from *S. hygroscopicus* AM-3672, identified employing DNA fragments from geldanamycin gene cluster as probes (A. RASCHER, Z. HU *et al.*, ms in preparation). Several genes coding for putative hydroxylases were found in the cluster, and it was not possible to determine from sequence comparisons which one encoded the C15 hydroxylase activity. To identify the C15-hydroxylase gene, a series of cosmids carrying the tailoring genes flanking the

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two sides of the herbimycin PKS genes were directly introduced into the geldanamycin producing strain–*S. hygroscopicus* NRRL3602[8,10]. None of recombinant strains produced 15-hydroxygeldanamycin. Instead two novel compounds (KOSN-1645 and KOSN-1646) were unexpectedly found in one of the recombinant strains, which still produced geldanamycin as the major product. Here we report the production and characterization of geldanamycin derivatives generated through the direct microbial bioconversion and genetic engineering.

Materials and Methods

Bacterial Strains, Plasmids and Growth Condition

The geldanamycin-producing strain, first described by DeBoer *et al.*[10,11] as *S. hygroscopicus* var. *geldanus* var. *nova* UC-5208, was obtained from the Northern Regional Research Laboratory as *S. hygroscopicus* NRRL 3602. *S. hygroscopicus* AM-3672[9] was a gift from Professor Satoshi Ōmura. R5 agar medium[12] was used to maintain both strains and their derivatives. Geldanamycin production medium (GPM)[11] was used as both seed and fermentation medium for both *S. hygroscopicus* NRRL 3602 and *S. hygroscopicus* AM-3672. Cultures were grown at 30°C for all experiments.

The organization of the herbimycin PKS gene cluster is very similar to that of the geldanamycin PKS gene cluster[8]. Cosmid pKOS279-78.4, which contains a *ca.* 44 kb DNA insert carries the loading module and the ketosynthase (KS) domain of the first module of the herbimycin gene PKS cluster and the large DNA segment (*ca.* 38 kb in size) upstream of the loading module, and the immediate upstream region that encodes two monooxygenases (homologues of GdmL[8] and PikC[13]. A. Rascher, Z. Hu *et al.*, ms in preparation and Rascher *et al.*[8]). pKOS279-78.4 does not bear any sequences that could encodes for any methylase or enzymes involving in the biosynthesis of 6-methyl-3-amino-5-hydroxybenzote, which might be involved in the production of KOSN-1645 and KOSN-1646. This cosmid carries the *attP* site and the integrase gene of phage ϕC31 and apramycin resistance gene from pSET152[14] (so that it can be integrated into the chromosomal *attB* site after entry into the *S. hygroscopicus* host, *via* conjugation from *Escherichia coli*[15]).

Bioconversion

R5 plates inoculated with *S. hygroscopicus* AM-3672 were incubated for about two weeks until sporulation was apparent. A segment of the agar from a sporulating plate was used to inoculate 50 ml of medium in a 250 ml flask. The flask was grown on a shaker for about three days and the culture broth was then transferred into 500 ml medium in a 2.8-liter Fernbach flask. This was incubated for one day and used as seed culture inoculate 20-liter or 5-liter fermenters.

The bioconversion experiments were carried out in a 5-liter fermenter (B. Braun MD5) with a 4.5-liter working volume and two 20-liter fermenters (New Brunswick Scientific BioFlo IV) with an 11-liter working volume. The pH was controlled automatically at 6.5 with 2.5 N sulfuric acid and 2.5 N sodium hydroxide. Dissolved oxygen was controlled at above 30% level with agitation (200～315 RPM for the 20-liter and 600～700 RPM for the 5-liter). Pressure was set at 0.25 bar in the 20-liter fermenters. The 5-liter fermenter was operated without backpressure. Airflow was set at 0.45 vvm and 0.9 vvm in the 20-liter and 5-liter fermenters, respectively. Foam was controlled by automatic addition of Antifoam B (JT Baker). Fermenters were started with inoculation of 5% of seed culture and 500 mg/liter geldanamycin (in DMSO) was added after 24 hours. Fermentation broth was harvested 2～5 days after geldanamycin feeding and was kept frozen.

Purification of 15-Hydroxylgeldanamycin, KOSN-1633 and Methyl Geldanamycinate

Frozen fermentation broth was thawed at room temperature over 24 hours. Methanol (30% v/v in water) and diatomaceous earth (Celite Corp., Lompoc, CA) (approximately 25 ml/liter broth were added, and the resulting mixture was allowed to stand for 5～10 minutes before filtering through Whatman #4 filter paper in a Buchner funnel. The clarified broth was then loaded onto a column of HP20 resin (4.8×50 cm, 900 ml) that had been washed with methanol and equilibrated in 30% methanol. The column was washed with 30% methanol, and then eluted with 100% methanol. The methanol pool was then dried to solids by rotary evaporation.

Water (150 ml) and ethyl acetate (200 ml) were added to the solid product pool, after combining thoroughly, the mixture was transferred to a separatory funnel, and the aqueous portion was extracted with another 2×100 ml of ethyl acetate. The ethyl acetate extracts were combined and washed with 2×100 ml water. At this point, a large amount of solid material remained on the glassware; these solids were re-suspended in methanol and the supernatant was analyzed by HPLC. Approximately half the 15-hydroxygeldanamycin was found in this re-dissolved solids portion, while the other half was in the ethyl acetate extract.

The ethyl acetate extract was evaporated and combined

with the dissolved solids (total volume 700 ml) and diluted to 50% methanol. The resulting solution was loaded onto a column of HP20-SS resin, washed with 50% methanol, and eluted with a stepped gradient. 15-hydroxygeldanamycin eluted in 65% methanol. The fraction eluting in 55~60% methanol was evaporated and set aside for isolation of a minor product (see below).

Fractions containing 15-hydroxygeldanamycin were pooled, and C18 chromatography was performed on a 4.8×25 ml column with 40% methanol as the loading solvent and 55% methanol as the eluting solvent. Fractions containing 15-hydroxygeldanamycin were pooled, and the methanol was removed by rotary evaporation. The resulting aqueous mixture was then extracted with 100 ml ethyl acetate followed by 2×100 ml dichloromethane. The organic portions were pooled and evaporated to yield 200 mg solids. The purified products appeared to decompose partially when re-dissolved in methanol as shown by LC-MS.

The C18 chromatography step removed a peak with an HPLC retention time of 6.9~7.1 minutes that had been increasing in size throughout the purification. However, upon drying and re-dissolving a sample of the product, it was apparent that this peak had reappeared. A 20 mg sample of material was purified by preparative HPLC, which separated the two peaks. LC-MS analysis indicates that the "impurity peak" has a molecular weight two mass units less than that of 15-hydroxygeldanamycin. After the HPLC-purified 15-hydroxygeldanamycin was evaporated and re-dissolved in methanol, it again appeared as a mixture.

For isolation of a minor analog (KOSN-1633), the fraction eluting from HP20-SS in 55~60% methanol (see above) was dissolved in 30% aqueous methanol and fractionated by C18 chromatography on a 4.8×25 cm column of Baker-Bond 40 μm prep LC resin that had been washed with methanol and equilibrated in 30% aqueous methanol. The column was loaded at 250 cm/hour using 30% methanol, then eluted at 150 cm/hour with a step gradient from 30~100% methanol. Fractions were analyzed by HPLC, and those eluting in 55% methanol were pooled and evaporated. The residue was re-dissolved in 40% aqueous acetonitrile, filtered, and purified by preparative HPLC using an InertSil ODS 8 μm 30×250 mm column (MetaChem) using 40% acetonitrile at 35 ml/minute, with UV detection at 300 nm. Fractions corresponding to the major peak, eluting at ~15 minutes, were pooled and the organic solvent was removed by rotary evaporation. The aqueous residue was extracted with ethyl acetate, which was then evaporated to dryness to yield 50 mg of solid.

In a separate bioconversion culture with shaking flasks, methyl geldanamycinate (10 mg/liter) was isolated with a similar procedure as used for purification of 15-hydroxygeldanamycin.

Introduction of pKOS279-78.4 into *S. hygroscopicus* NRRL 3602 and Isolation of KOSN-1645 and KOSN-1646

Cosmid-pKOS279-78.4 was introduced into *S. hygroscopicus* NRRL 3602 by conjugation from an *E. coli* ET12567/pUB307 host strain according to a published method[15]. The new strain, named K279-78, was fermented in 1 liter of GDM medium for about 6 days. Two compounds KOSN-1645 and KOSN1646 were purified from the fermentation broth with the same procedures used for purification of 15-hydroxygeldanamycin. The purifications of KOSN-1645 and KOSN-1646 were similar to that of 15-hydroxygeldanamycin.

Cytotoxicity Assay of Geldanamycin Analogs against Human Breast Cancer Cell Line

All compounds to be assayed for cytotoxicity were formulated in DMSO (Sigma Chemical Co., St. Louis, MO) as 10 mM stock solutions and stored at −20°C. The human breast cancer cell line SKBr3 was obtained from the American Type Culture Collection (ATCC, Rockville, MD) and cultured in McCoy'5A modified medium supplemented with 10% Fetal Bovine Serum (Hyclone, Logan, UT) and 2 nM L-glutamine. The tumor cells were maintained in a 37°C, 5% CO_2 humidified incubator. For cytotoxicity assays, 4000 SKBr3 cells were plated in 50 μl per well into 96-well microtiter black plates. Cells were allowed to adhere for 24 hours. Each compounds ranging from 0.001 to 10000 nM in 50 μl was added to cells in duplicate wells. After 72 hours incubation, the plates were placed at room temperature for 30 minutes, 100 μl of CellTilter-Glo Luminescent Reagent (Promega, Madison, WI) were added to each well, the contents were mixed for 5 minutes and the plates kept at room temperature for 20 minutes. Luminescences was recorded with a Wallac Victor^2 Multilable Counter (PerkinElmer, Downers Grove, IL) and IC_{50} values were determined using Kaleidagraph.

Results and Discussion

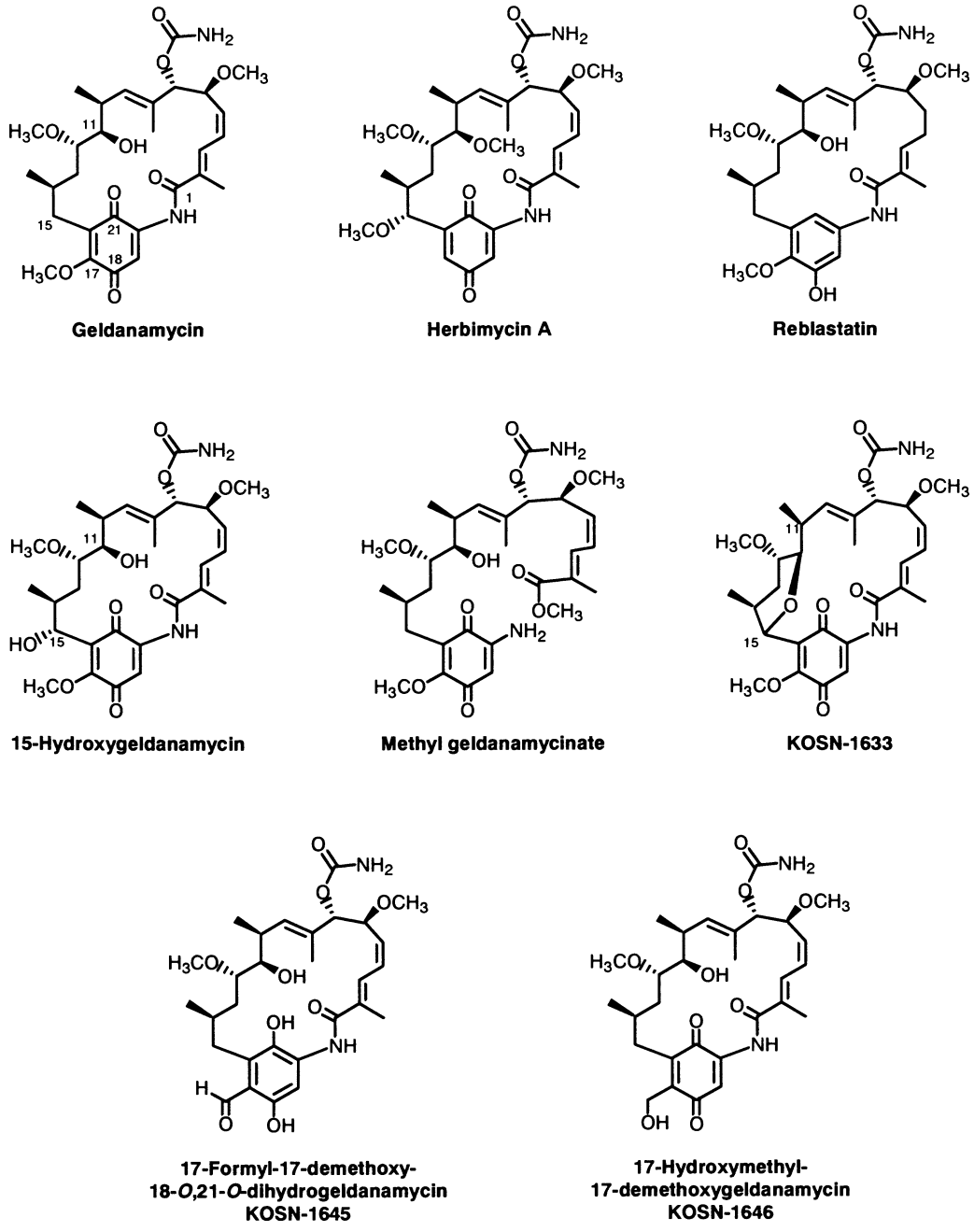
Microbial Conversion of Geldanamycin to 15-Hydroxygeldanamycin

The herbimycin-producing strain, *S. hygroscopicus* AM-3672, was used in the hope that it would oxidize

geldanamycin at its C15 position to accomplish the direct microbial conversion. As anticipated, 15-hydroxygeldanamycin was formed as the major product when geldanamycin was added to the fermentation with *S. hygroscopicus* AM-3672. A minor compound, a tricyclic geldanamycin (KOSN-1633) analog was also isolated from the microbial bioconversion. The structure of KOSN-1633 implied that it was resulted from the cyclization between C15-OH and C11-OH and along with 17-*O*-demethylation of 15-hydroxygeldanamycin. The successful addition of the C15-hydroxyl to geldanamycin indicated that the C15-methoxy group in herbimycin A was formed through hydroxylation followed by *O*-methylation. Occasionally in the samples taken from flask fermentations, there was a small LC-MS peak of *m*/*z* 613 [M+Na] corresponding to the mass of 15-methoxygeldanamycin. Unfortunately, We could not isolate enough material to identify the compound.

Another compound, a methylated derivative of geldanamycin arising from a test experimental microbial bioconversion in shake flask experiments with

Fig. 1. Structures of geldanamycin, herbimycin A, reblastatin, methyl geldanamycinate and four newly discovered geldanamycin analogs.



S. hygroscopicus AM-3672 was also isolated. Methyl geldanamycinate was previously synthesized by treating geldanamycin with potassium carbonate in boiling methanol[1]. Since the methanolysis does not happen at ambient temperature, it is reasonable to assume that the methyl geldanamycinate isolated here was formed during the bioconversion.

Characterization of 15-Hydroxygeldanamycin and Methyl Geldanamycinate

High-resolution MS measurements for 15-hydroxy-geldanamycin (Fig. 1) were consistent with a formula of $C_{29}H_{40}N_2O_{10}$ for a monohydroxylated geldanamycin analogue. Its ^1H and ^{13}C NMR were similar to those of geldanamycin (Tables 1 and 2). However, signals for C-15 methylene were absent in its ^1H NMR; instead a new doublet at 4.58 ppm for a methine group was observed. ^1H and ^{13}C chemical shifts (except for the quaternary carbons) were assigned from multiplicity-edited HSQC and COSY. Chemical shifts for the quaternary carbons were assigned by comparison with those of geldanamycin. The stereochemistry of C-15 was assumed to be the same as in herbimycin A.

High-resolution MS measurements for methyl geldanamycinate (Fig. 1) were consistent with a formula of $C_{30}H_{44}N_2O_{10}$. Its ^1H and ^{13}C NMR were obviously different from those of geldanamycin in the olefinic region (Tables 1 and 2). An extra methoxyl was observed, with 3.76 ppm and 52.58 ppm for ^1H and ^{13}C NMR respectively. All chemical shifts were assigned from multiplicity-edited HSQC, COSY and HMBC.

Characterization of KOSN-1633

A molecular formula of $C_{28}H_{36}N_2O_9$ was established for

Table 1. ^{13}C NMR chemical shifts for geldanamycin-derivatives characterized in this study.

	15-OH GDM ($CDCl_3$)	Me GDMate (CD_3OD)	KOSN 1633 ($CDCl_3$)	KOSN 1645 ($CDCl_3$)	KOSN 1646 ($CDCl_3$)
1	167.9	170.1	167.4	167.3	168.4
2	134.5	131.0	133.5	136.7	134.7
2-Me	12.4	12.7	11.8	12.4	12.8
3	127.5	133.3	129.6	124.7	127.4
4	125.9	129.2	125.5	127.1	126.2
5	138.5	135.1	139.3	132.9	136.8
6	82.8	79.0	83.7	80.9	81.1
6-OMe	57.8	57.1	58.0	56.9	56.7
7	82.6	81.3	81.9	82.8	81.5
7-carbamate	155.9	158.9	156.0	156.5	155.9
8	134.2	132.2	132.6	133.4	132.4
8-Me	12.3	14.8	12.7	12.6	12.9
9	131.9	133.7	132.0	133.4	132.4
10	31.7	36.0	32.2	32.3	32.3
10-Me	11.7	17.6	12.2	12.1	12.5
11	72.8	75.4	74.1	74.7	73.0
12	80.4	81.9	75.8	80.3	80.8
12-OMe	56.9	57.1	56.2	57.1	57.4
13	30.0	36.2	35.3	35.4	28.5
14	33.0	30.6	27.2	29.2	34.4
14-Me	18.6	20.4	75.6	22.4	22.5
15	72.7	32.1	75.6	33.4	35.5
16	127.3	127.8	116.6	127.3	138.6
17	156.2	160.1	153.4	113.1	142.5
17-X	62.0	62.0	--	193.2	57.7
18	184.3	185.5	182.6	159.4	189.4
19	111.4	98.7	106.8	104.7	113.6
20	138.1	151.0	141.2	135.6	141.4
21	183.9	183.7	182.1	136.4	184.9
1-OMe	--	52.9			

Note: 15-OH GDM: 15-hydroxygeldanamycin; Me GDMate: Methyl geldanamycinate.

Table 2. ^{1}H NMR chemical shifts for geldanamycin-derivatives characterized in this study.

	15-OH GDM ($CDCl_3$)	Me GDMate (CD_3OD)	KOSN 1633 ($CDCl_3$)	KOSN 1645 ($CDCl_3$)	KOSN 1646 ($CDCl_3$)
2-Me	2.02 (s)	1.94 (s)	1.98 (s)	1.59 (s)	2.02 (s)
3	6.90 (d,11.2)	7.52 (d,12.0)	7.02 (d, 11.5)	6.85 (d, 11.2)	6.96 (d, 11.2)
4	6.53 (t,11.8)	6.64 (t,11.6)	6.47 (t, 11.5)	6.35 (t, 11.2)	6.56 (t, 11.4)
5	5.95 (dd, 11.2, 8.8)	5.61 (t, 10.4)	5.87 (dd, 11.0, 7.5)	5.71 (t, 10.4)	5.87 (t, 4.4)
6	4.30 (d, 8.4)	4.35 (dd, 9.6, 7.2)	4.37 (d, 7.5)	4.31 (d, 10.4)	4.33 (d, 8.4)
6-OMe	3.37 (s)	3.27 (s)	3.37 (s)	3.36 (s)	3.35 (s)
7	5.02 (s)	4.94 (d, 7.2)	5.00 (s)	4.96 (s)	5.21 (s)
8-Me	1.78 (s)	1.64 (s)	1.73 (s)	1.79 (s)	1.77 (s)
9	5.60 (d, 10.0)	5.36 (d, 10.0)	5.46 (d, 9.0)	5.94 (d, 9.6)	5.74 (d, 10)
10	2.86 (m)	2.36 (m)	2.70 (m)	2.81 (qn, 7.6)	2.75 (m)
10-Me	0.85 (d, 6.8)	0.96 (d, 6.8)	0.85 (d, 6.5)	0.93 (d, 7.2)	0.9 - 1.0 (ov)
11	3.30 (m)	3.52 (dd, 9.2, 2.4)	4.13 (d, 9.5)	3.63 (m)	3.47 (m)
12	3.20 (m)	3.16 (d, 10.8)	3.40 (m)	3.5 (m)	3.4 (m)
12-OMe	3.35 (s)	3.31 (s)	3.36 (s)	3.22 (s)	3.32 (s)
13	1.70 (m)	1.58 (m)	1.58 (m)	1.88 (br d, 14)	1.6 - 1.8 (ov)
		1.02 (m)	1.91 (br d, 1.4)	1.75 (ov)	
14	2.11 (m)	1.84 (m)	2.25 (m)	1.6 - 1.8 (ov)	1.6 - 1.8 (ov)
14-Me	1.18 (d, 6.4)	0.79 (d, 6.8)	0.84 (d, 5.0)	0.95 (d, 8.8)	0.9 - 1.0 (ov)
15	4.58 (d, 8.0)	2.29 (m)	4.51 (d,11)	2.72 (br d, 14)	2.41 (dd, 10, 12)
				2.93 (dd, 11.2, 14)	2.64 (br d, 10)
17-X	4.16 (s)	4.02 (s)	--	10.09 (s)	4.52 (dd, 7.2, 12)
					4.64 (dd, 5.8, 12)
19	7.30 (s)	5.47 (s)	7.23 (s)	7.93 (s)	7.46 (s)
22 (NH)	8.66 (s)	--	9.22 (br s)	8.73 (s)	8.63 (s)
18-OH	--	--	--	12.11 (s)	
21-OH	--	--	--	8.65 (br s)	
17-CH_2OH					2.85 (br s)

KOSN-1633 (Fig. 1) from ^{13}C NMR spectrum and high-resolution mass spectral data (ESI TOF MS *m/z* 567.2303, calculated for $C_{28}H_{36}N_2O_9Na$ ($[M+Na]^+$) 567.2313). Carbon-hydrogen connectivity was established using the multiplicity-edited HSQC spectrum, while gsCOSY and ctHMBC data allowed tracing of the carbon–oxygen skeleton. The molecular formula indicates that KOSN-1633 contains one carbon fewer than geldanamycin, and the proton NMR spectrum lacks the 17-methoxy signal displayed by geldanamycin (δ 4.12). The molecular formula also indicates an additional element of unsaturation with respect to geldanamycin, which is accounted for by a cyclic ether joining carbons 11 and 15. The location of this ether linkage is confirmed by an HMBC connecting H-15 (δ 4.51) and C-11 (δ 7.41). One- and two-dimensional NMR data are consistent with the rest of the structure being identical to geldanamycin.

Characterization of KOSN-1645 (17-Formyl-17-demethoxy-18-*O*, -21-*O*-dihydrogeldanamycin) and KOSN-1646 (17-Hydroxymethyl-17-demethoxygeldanamycin)

KOSN-1645 and -1646 are related compounds and minor products, produced by *S. hygroscopicus* K279-78. K279-78 mainly produced herbimycin, whose synthesis starts with AHBA (3-amino-5-hydroxybenzoic acid). It is not known if AHBA is modified at C6 (methylation or methoxylation) before it is used as the starter or after the full polyketide backbone is produced, to generate the compounds KOSN-1645 and KOSN-1646. The finding of these two compounds is surprising. *S. hygroscopicus* AM-3672, the herbimycin-producing strain, has not been reported to produce any analog with a 17-methyl group. We cannot explain how these two compounds formed. A hydroxylase must be involved in the biosynthesis of the compounds, but it is not clear if the activity is encoded by the genes on the plasmid introduced into the geldanamycin-producing host.

Table 3. Cytotoxicity of geldanamycin analogues against SKBR3 cell line.

Compounds	SKBr3 IC_{50} (nM)
Geldanamycin	37
Herbimycin A	160
Reblastatin	600
15-hydroxygeldanamycin	710
KOSN-1633	1533
KOSN-1645	2559
KOSN-1646	3163

Further study is needed to find out how the compounds are formed.

The formula $C_{29}H_{40}N_2O_9$ of the two isomers KOSN-1645 and -1646 was established by LC-MS and HRMS. Comparison of the NMR spectra of KOSN-1645 in $CDCl_3$ with the spectra of geldanamycin revealed that the 17-MeO is missing, while several peaks downfield from δ 8 ppm were present. One peak at δ 193.2 ppm suggested the presence of one extra carbonyl group. Carbon–hydrogen connectivity was established using the multiplicity-edited HSQC spectrum, while gsCOSY and HMBC spectra allowed tracing of the carbon-oxygen skeleton. The ^{13}C and ^{1}H chemical shifts are summarized in Tables 1 and 2, respectively. ESI TOF MS *m/z* 583.2656, calcd for $C_{29}H_{40}N_2O_9$ ($[M+Na]^+$) 583.2626.

KOSN-1646 was characterized in a 65 : 35 mixture with KOSN-1645. The ^{1}H and ^{13}C chemical shifts were assigned by comparison with spectra of KOSN-1645 and geldanamycin and are summarized in Table 1 and Table 2. ESI TOF MS *m/z* 583.2646, calcd for $C_{29}H_{40}N_2O_9$ ($[M+Na]^+$) 583.2626.

Biological Activity

The cytotoxicity of 15-hydroxygeldanamycin is almost equal to that of reblastatin and 20 times lower than that of geldanamycin. KOSN-1633 is two-fold less cytotoxic than 15-hydroxygeldanamycin (Table 3). However, the introduction of hydroxyl group into C15 position of geldanamycin makes it possible to generate novel derivatives through chemical modification. Methyl geldanamycinate was reported to have much lower activity (200～500×) and lost activity in cell assay[1]. KOSN-1645 and KOSN-1646 showed significantly reduced or no cytotoxicity against SKBr3 cells.

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