Note

GLIDOBACTINS D, E, F, G AND H; MINOR COMPONENTS OF THE ANTITUMOR ANTIBIOTIC GLIDOBACTIN

MASAHISA OKA, HIROAKI OHKUMA, HIDEO KAMEI, MASATAKA KONISHI, TOSHIKAZU OKI and HIROSHI KAWAGUCHI

Bristol-Myers Research Institute,
Tokyo Research Center,
2-9-3 Shimo-meguro, Meguro-ku,
Tokyo 153, Japan

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Glidobactin is a complex of novel antitumor antibiotics elaborated by *Polyangium brachysporum* sp. nov. No. K481-B101 (ATCC 53580). The three major components, glidobactins A, B and C have been isolated and their chemical and biological properties characterized[1]. They are unique acylpeptides structurally unrelated to any previously known antibiotics.

In our further investigation of the metabolites, five related congeners named glidobactins D, E, F, G and H were found as co-products of the major glidobactins A, B and C. Glidobactins D, E and F differ from glidobactin A only in their fatty acid side chains, while glidobactin G contains a hydroxymethyl group on the 12-membered core ring in place of the methyl group of the other glidobactins. The structure of glidobactin H has not yet been determined. This communication presents the isolation, properties, structures and biological activities of the new components.

The fermentation of *P. brachysporum* sp. nov. No. K481-B101 and isolation of the crude glidobactin solid were carried out as described before[1]. A portion of the solid (114 g) was loaded on a column of a reversed phase silica (Merck Kieselgel 60 silanised, 3.5 liters) which had been equilibrated with 70% aqueous methanol. Elution was carried out with 70% and 80% aqueous methanol and the eluates were pooled on the basis of the bioassay and HPLC (SSC-ODS-262, 80% aqueous methanol elution). Evaporation of the first pool yielded 16.3 g of semi-pure solid which contained glidobactins D, E, F, G and H. The work-up of the second and third pools afforded pure glidobactin A (14.6 g) and a mixture of glidobactins A, B and C (44.6 g), respectively.

The mixture of glidobactins D, E, F, G and H (12.7 g) was chromatographed on a column of silica gel (Wakogel C-200, 700 ml) developing with $CHCl_3$ - MeOH (10:1 and 5:1). The eluate was collected in fractions (18 ml each) which were analyzed by TLC (Merck Kieselgel $60F_{254}$, EtOH - H_2O, 55:45). The initial half of the active fractions (No. 91~160) was pooled, concentrated and lyophilized to afford 1.2 g of white solid which contained glidobactins F and H. The latter half was similarly worked up to give 930 mg of glidobactins D, E and G mixture. The glidobactins F and H complex (1.2 g) was chromatographed on reversed phase silica gel (160 ml) with MeOH - H_2O (1:1, 1 liter and 5:1, 2.5 liters). The first bioactive fractions were pooled, evaporated *in vacuo*, and lyophilized to give pure glidobactin F (131 mg). The second active fractions were evaporated to give a solid (88 mg) which was again purified by silica gel chromatography (column: 50 ml, elution: $CHCl_3$-MeOH) to isolate homogeneous glidobactin H (1.9 mg).

The complex of glidobactins D, E and G (930 mg) obtained above was again chromatographed on a silica gel column (Wakogel C-200, 190 ml). The elution was carried out by a mixture of $CHCl_3$ - MeOH (10:1, 1 liter and 5:1, 1.6 liters) and the active eluates were pooled and concentrated to give a purer solid of glidobactins D, E and G complex (97 mg). This complex was separated by reversed phase silica gel chromatography with aqueous methanol (50~70%) elution. The first active fractions were pooled and evaporated to give pure glidobactin D (8.4 mg). The second and the third active fractions were similarly worked up to isolate pure glidobactins E (5.1 mg) and G (18.1 mg), respectively.

The physico-chemical properties of glidobactins D, E, F, G and H are similar to those of the major components, glidobactins A, B and C. They were readily soluble in methanol, butanol and dimethyl sulfoxide, slightly soluble in chloroform, acetonitrile and ethyl acetate but insoluble in hexane and water. They were posi-

Table 1. Physico-chemical properties of glidobactins D, E, F, G and H.

	D	E	F	G	H
Nature	White powder	White powder	White powder	White powder	White powder
MP (°C, dec)	204	195	233	217	191
Molecular formula	$C_{27}H_{44}N_4O_7$	$C_{27}H_{44}N_4O_7$	$C_{25}H_{40}N_4O_6$	$C_{27}H_{44}N_4O_7$	—
UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$)	257 (662)	260 (532)	260 (474)	257 (668)	260 (410)
IR ν_{max}^{KBr} cm^{-1}	3300, 1640, 1530	3300, 1640, 1530	3300, 1640, 1530	3300, 1640, 1530	3300, 1620, 1530
TLC[a] (Rf)	0.68	0.61	0.50	0.43	0.59
HPLC[b] (Rt; minutes)	2.2	2.6	2.8	4.4	2.4

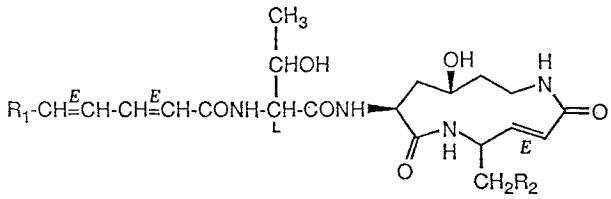
[a] Silanized, plate: EtOH - H_2O (55:45).
[b] SSC-ODS-262, Rt: retention time, MeOH - H_2O (4:1).

tive to Ryodon-Smith, iodine and sulfuric acid reactions but negative to ninhydrin, Sakaguchi, anthrone and Dragendorff tests. The molecular formulae of glidobactins D, E and G were determined to be $C_{27}H_{44}N_4O_7$, and glidobactin F as $C_{25}H_{40}N_4O_6$, by their mass and ^{13}C NMR spectra. The spectral data of glidobactin H did not allow assignment of molecular formula. The UV spectra of the five new components showed a single absorption band at 260 nm suggesting an $\alpha,\beta,\gamma,\delta$-unsaturated carbonyl function common to glidobactins A, B and C. Their IR spectra indicated the amide carbonyl absorption at 1640 and 1530 cm^{-1}.

The physico-chemical properties obtained for glidobactins D, E, F, G and H indicated that they were closely related to the major glidobactin components. The molecular formulae assigned to glidobactins D, E and G ($C_{27}H_{44}N_4O_7$) demonstrated that they are mono-hydroxy analogs of glidobactin A ($C_{27}H_{44}N_4O_6$). Glidobactin F exhibited a C_2H_4-fewer molecular formula than that of glidobactin A. Each of the five components was hydrolyzed with 6 N HCl at 110°C for 16 hours in a sealed tube and the hydrolysate examined by TLC (BuOH - AcOH - H_2O, 3:1:1, ninhydrin detection). Glidobactins D, E, F and H afforded the same amino acids (threonine, (*E*)-4-amino-2-pentenoic acid and *erythro*-4-hydroxylysine) as glidobactin A indicating that they differed from the latter component only at the fatty acid moiety. All of these fatty acids were considered to be $\alpha,\beta,\gamma,\delta$-unsaturated acids based on their common UV maximum at 260 nm with the major antibiotics. The secondary ion mass spectrum (SI-MS) of glidobactin D yielded prominent fragment ions at *m/z* 195 (fatty acid), *m/z* 242 (glidobamine)[2] and *m/z* 296 (fatty acid-threonine) along with a protonated molecular ion at *m/z* 537. The identical molecular and fragment ions were observed in SI-MS of glidobactin E. The fatty acids from glidobactins D and E were extracted from their hydrolysates with ethyl acetate and treated with diazomethane to afford methyl esters ES-1 (from glidobactin D) and ES-2 (from glidobactin E), both of which yielded a protonated molecular ion at *m/z* 227. Since the ^1H NMR spectra had revealed that all the fatty acids of glidobactin components were straight chain acids, the acids of glidobactins D and E must be a mono-hydroxy-2,4-dodecadienoic acid. Seven sp^3 carbon signals of the fatty acid moiety of glidobactin A have been assigned as CH_3 (δ 13.4 ppm)-CH_2 (21.6)-CH_2 (30.8)-CH_2 (28.1)-CH_2 (28.0)-CH_2 (28.0)-CH_2 (31.8)[3]. A prediction of carbon shifts in substituted alkanes is well-established[4], and is used for the determination of the position of a substituent. Based on the chemical shifts of glidobactin A, calculation of the shift values of a hydroxyl group allowed us to assign the following partial structures to the fatty acids of glidobactins D and E; glidobactin D, CH_3 (δ 9.5)-CH_2 (29.5)-CHOH (70.7)-CH_2 (36.1)-CH_2 (24.5)-CH_2 (28.4)-CH_2 (31.9) and E, CH_3 (δ13.4)-CH_2 (21.7)-CH_2 (31.0)-CH_2 (24.4)-CH_2 (36.4)-CHOH (69.2)-CH_2 (40.4).

The electron impact mass spectrum (EI-MS) of glidobactin F did not give the molecular ion but showed a strong fragment ion (base peak) at *m/z* 151 which was derived from the fatty acid. Acetylation of glidobactin F in acetic anhydride and pyridine gave a diacetate derivative which gave the molecular ion at *m/z* 576 along with the fragment ions at *m/z* 151 (fatty acid) and *m/z* 285 (acetylglidobamine). Thus, glidobactin F

Fig. 1. Structures of glidobactins D, E, F and G.



Glidobactin D	$R_1=CH_3CH_2\underset{\underset{OH}{\vert}}{CH}(CH_2)_4-$	$R_2=H$
Glidobactin E	$R_1=CH_3(CH_2)_4\underset{\underset{OH}{\vert}}{CH}CH_2-$	$R_2=H$
Glidobactin F	$R_1=CH_3(CH_2)_4-$	$R_2=H$
Glidobactin G	$R_1=CH_3(CH_2)_6-$	$R_2=OH$
Glidobactin A	$R_1=CH_3(CH_2)_6-$	$R_2=H$

Table 2. Antifungal activity of glidobactin components.

	MIC (μg/ml, SABOURAUD glucose agar)					
	D	E	F	G	H	A
Candida albicans IAM 4888	>50	>50	50	25	>50	3.1
C. albicans A9540	>50	>50	12.5	25	>50	1.6
Cryptococcus neoformans D 49	>50	>50	50	25	>50	25
Aspergillus fumigatus IAM 2530	>50	>50	25	12.5	>50	1.6
A. flavus FA 21436	>50	>50	>50	50	—	25
Fusarium moniliforme A 2284	>50	>50	>50	>50	>50	>50
Trichophyton mentagrophytes D 155	>50	>50	>50	25	>50	25

was assigned as the decadienoyl analog. The fatty acid of glidobactin H could not be determined since the antibiotic did not exhibit the molecular ion or diagnostic fragment ions in the mass spectrometry.

It has been demonstrated by the molecular formula that glidobactin G possessed an additional hydroxyl group over glidobactin A. The acid hydrolysate of glidobactin G contained threonine, *erythro*-4-hydroxylysine and an unidentified amino acid but no trace of (*E*)-4-amino-2-pentenoic acid indicating that glidobactin G differed from the other components at the (*E*)-4-amino-2-pentenoic acid moiety. This was collaborated by the ^1H NMR spectrum of glidobactin G which lacked the doublet methyl signal at δ 1.24 ppm attributable to (*E*)-4-amino-2-pentenoic acid. Upon enzymatic hydrolysis with papain[3], glidobactin G afforded an acidic, lipophilic substance (AC-1) and a basic, water soluble substance (BW-1). AC-1 was identical with *N*-(*E*,*E*)-2,4-dodecadienoyl-L-threonine by comparison with an authentic sample obtained from glidobactin A. The chemical ionization mass spectrum (CI-MS) of BW-1 showed the protonated molecular ion at *m*/*z* 258, 16 mass units higher than the corresponding moiety of glidobactins A, B and C. Thus, glidobactin G contains (*E*)-4-amino-5-hydroxy-2-pentenoic acid in place of the (*E*)-4-amino-2-pentenoic acid of glidobactin A. The structures of glidobactins D, E, F and G are shown in Fig. 1.

All five new components of glidobactin did not exhibit significant activity against Gram-positive and Gram-negative bacteria. Glidobactins F and G were slightly active against fungi but activity was much weaker than that of glidobactin A (Table 2).

Antitumor activity of glidobactins D, E, F, G and H was examined for mouse P388 leukemia following the method described in the previous report.[1] Glidobactins F and G showed significant prolongation of the life span at 3 mg/kg/day but the other three components were inactive at the doses tested (0.3~3 mg/kg/day). The antitumor activity of glidobactins F and G

was weaker than that of glidobactin A which was active at 0.3 and 1 mg/kg/day.

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