CHEMICAL MODIFICATION OF THE ANTITUMOR ANTIBIOTIC GLIDOBACTIN

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(Received for publication July 22, 1988)

A variety of glidobactin analogs modified at the fatty acid, L-threonine and nucleus moieties of the molecule were synthesized and their structure-activity relationships examined. The antitumor and antifungal activity was greatly influenced by modification of the fatty acid glidobactin, with the dodecanoyl and tetradecanoyl analogs exhibiting better antitumor activity than the parent antibiotics. Replacement of the L-threonine with other amino acids greatly reduced the activity and reduction of the double bond of the nucleus completely eliminated the biological activity of glidobactin.

Glidobactin is a complex of new antitumor antibiotics produced by *Polyangium brachysporum* No. K481-B101 (ATCC 53080)[1]. The three components, glidobactins A, B and C were isolated and their unique acylpeptide structures were elucidated by a combination of chemical and enzymatic degradation and spectral analysis[2]. The glidobactins inhibit growth of fungi and tumor cells and markedly prolong the life span of mice implanted with P388 leukemia. The strong antitumor activity and unusual structures of glidobactin prompted us to modify the antibiotics. Upon treatment with papain or ficin, glidobactin A (**1a**) was cleaved to give the acyl-L-threonine (**2**) and the cyclic amine (glidobamine, **3**) (Scheme 1). On the other hand, the acylase of *Pseudomonas* sp. hydrolyzed **1a** to yield (*E,E*)-2,4-dodecadienoic acid (**4**) and L-threonylcyclic amine (glidobactamine, **5**)[3]. These hydrolysis products provided appropriate starting materials for modification of the antibiotic. We report here the preparation, physico-chemical properties and biological activity of 32 glidobactin derivatives.

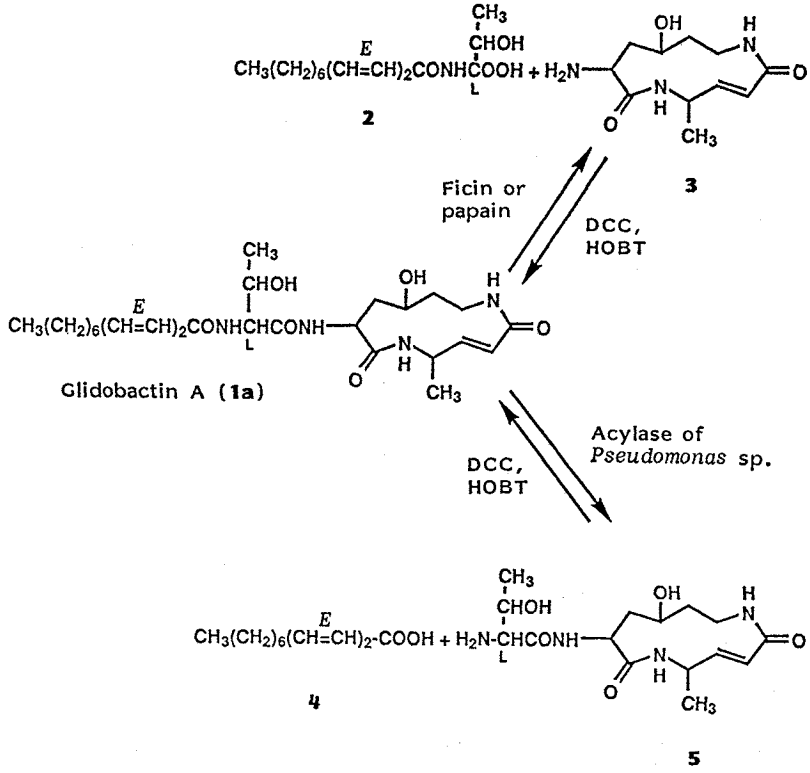
Chemistry

Modification of the fatty acid moiety of glidobactin was carried out by two routes (Scheme 2). Acylation of **5** with appropriate active esters, anhydrides or halides afforded glidobactin acyl analogs in good yields (Scheme 2, route a). Alternatively, coupling of appropriate acid with L-threonine by active ester method yielded acyl-L-threonines which were condensed with **3** to afford the fatty acid derivatives of glidobactin (route b).

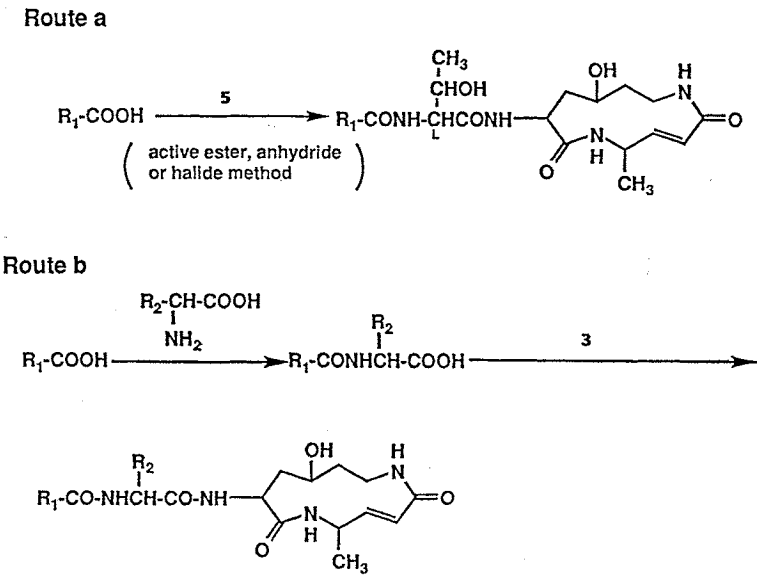
Replacement of the L-threonine with other amino acids was carried out by route b. The appropriate amino acids were acylated with **4** and the resulting (*E,E*)-2,4-dodecadienoylamino acids were then coupled with **3** to give the amino acid derivatives. For L-lysine and L-glutamic acid derivatives, the ω-amino and ω-carboxyl groups were protected with benzyloxycarbonyl and methyl groups, respectively and after condensation, were deblocked by HBr - AcOH or KOH to yield the free amino and acid form derivatives.

A direct acylation of **3** with **4** gave (*E,E*)-2,4-dodecadienoylglidobamine (**35**), which was distinguished from other derivatives in lacking the linker amino acid. Compound **30** which has an extra L-threonine between **4** and **5** was prepared by acylation of **5** with **2**.

Scheme 1. Enzymatic cleavage of glidobactin A (1a).

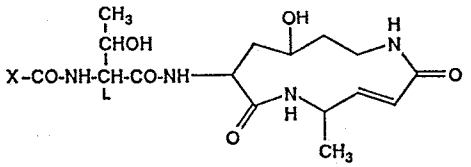


Scheme 2. Preparation of glidobactin derivatives.

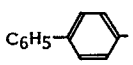
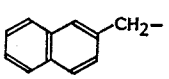
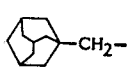
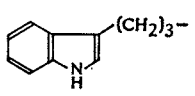
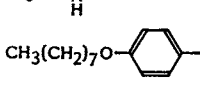


Catalytic hydrogenation of **3** with 10% palladium on charcoal yielded dihydroglidobamine (**36**). Coupling of **36** with **2** gave a dihydro-derivative of glidobactin A (**37**). Physico-chemical and spectral properties of the derivatives are illustrated in Tables 1~3.

Table 1. Physico-chemical properties of glidobactin derivatives **6**~**25**.

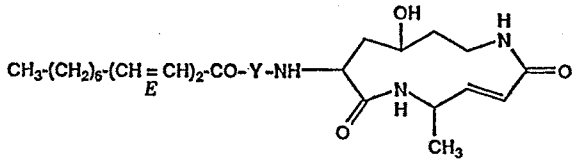


Compound	X	Route[a]	Yield[b] (%)	MP (°C)	UV λ_{max} nm ($E^{1\%}_{1cm}$)	Key signals in ^{1}H NMR (δ in ppm; 80 MHz, DMSO-d_6)	EI-MS (*m/z*)
6	CH_3–	a	98	248	End	0.99 (3H, d), 1.21 (3H, d), 1.89 (3H, s), 6.10 (1H, d), 6.40 (1H, dd), 7.35 (1H, t), 7.71 (1H, d), 7.80 (1H, d), 8.61 (1H, d)	385 ($M^+ + 1$)[c]
7	$CH_3(CH_2)_4$–	b	31	180	End	0.86 (3H, t), 1.02 (3H, d), 6.11 (1H, d), 6.40 (1H, dd), 7.40 (1H, t), 7.58 (1H, d), 7.61 (1H, d), 8.56 (1H, d)	440 (M^+)
8	$CH_3(CH_2)_6$–	b	37	232	End	0.86 (3H, t), 1.00 (3H, d), 6.11 (1H, d), 6.40 (1H, dd), 7.35 (1H, t), 7.57 (1H, d), 7.60 (1H, d), 8.56 (1H, d)	468 (M^+)
9	$CH_3(CH_2)_8$–	a	85	244	End	0.86 (3H, t), 1.02 (3H, d), 6.10 (1H, d), 6.40 (1H, d), 7.35 (1H, t), 7.58 (1H, d), 7.60 (1H, d), 8.56 (1H, d)	469 (M^+)
10	$CH_3(CH_2)_{10}$–	a	89	274	End	0.86 (3H, t), 1.02 (3H, d), 6.11 (1H, d), 6.41 (1H, dd), 7.36 (1H, t), 7.60 (2H, m), 8.55 (1H, d)	524 (M^+)
11	$CH_3(CH_2)_8CH\overset{E}{=}CH$–	a	83	232	211 (490)	0.86 (3H, t), 1.02 (3H, d), 6.05 (1H, d), 6.10 (1H, d), 6.40 (1H, dd), 6.50 (1H, m), 7.36 (1H, t), 7.66 (1H, d), 7.79 (1H, d), 8.55 (1H, d)	504 ($M^+ - H_2O$)
12	$HO(CH_2)_{11}$–	a	73	212	End	1.00 (3H, d), 6.10 (1H, d), 6.40 (1H, dd), 7.36 (1H, t), 7.60 (2H, m), 8.55 (1H, d)	525 ($M^+ - H_2O$)
13	$CH_3(CH_2)_{12}$–	a	94	239	End	0.86 (3H, t), 1.00 (3H, d), 6.10 (1H, d), 6.40 (1H, dd), 7.36 (1H, t), 7.58 (1H, d), 7.60 (1H, d), 8.56 (1H, d)	575 ($M^+ + Na$)[c]
14	$CH_3(CH_2)_{14}$–	b	18	216	End	0.86 (3H, t), 1.00 (3H, d), 6.10 (1H, d), 6.40 (1H, dd), 7.35 (1H, t), 7.60 (2H, br d), 8.56 (1H, d)	580 (M^+)

15	$CH_3(CH_2)_{10}(\overset{E}{CH}{=}CH)_2-$	a	29	232	260 (510)	0.86 (3H, t), 1.00 (3H, d), 6.20 (5H, m), 6.95 (1H, m), 7.35 (1H, t), 7.66 (1H, d), 7.88 (1H, d), 8.59 (1H, d)	336 (FA→Thr)[c]
16	$CH_3(CH_2)_{13}\underset{\displaystyle OH}{\underset{\vert}{CH}}-$	a	64	229	End	0.86 (3H, t), 1.00 (3H, d), 6.11 (1H, d), 6.40 (1H, dd), 7.35 (1H, t), 7.60 (2H, m), 8.58 (1H, d)	—
17	C_6H_5-	a	41	172	224 (248)	1.06 (3H, d), 1.22 (3H, d), 6.10 (1H, d), 6.40 (1H, dd), 7.40 (4H, m), 7.80 (4H, m). 8.57 (1H, d)	447 (M^++1)[c]
18	$C_6H_5CH_2-$	a	46	224	End	0.97 (3H, d), 1.21 (3H, d), 3.53 (2H, s), 6.11 (1H, d), 6.41 (1H, dd), 7.22 (5H, s), 7.35 (1H, t), 7.68 (1H, d), 7.95 (1H, d), 8.57 (1H, d)	461 (M^++1)[c]
19	$C_6H_5CH{=}CC_6H_5-$	a	85	165	220 (350), 281 (270)	0.98 (3H, d), 1.21 (3H, d), 6.10 (1H, d), 6.39 (1H, dd), 6.73 (1H, d), 6.9~7.6 (12H, m), 7.88 (1H, d), 8.56 (1H, d)	—
20		a	92	197	268 (690)	1.04 (3H, d), 1.24 (3H, d), 6.10 (1H, d), 6.40 (1H, dd), 7.3~8.3 (12H, m), 8.61 (1H, d)	523 (M^++1)[c]
21	$(C_6H_5)_2CH-$	a	74	235	End	0.95 (3H, d), 1.21 (3H, d), 5.22 (1H, s), 6.10 (1H, d), 6.40 (1H, dd), 7.22 (11H, m), 7.66 (1H, d), 8.11 (1H, d), 8.55 (1H, d)	
22		a	81	262	225 (1,700), 276 (19)	0.97 (3H, d), 1.21 (3H, d), 3.66 (2H, s), 6.10 (1H, d), 6.40 (1H, dd), 7.3~7.9 (9H, m), 8.05 (1H, d), 8.56 (1H, d)	511 (M^++1)[c]
23		a	80	196	End	1.02 (3H, d), 1.21 (3H, d), 1.94 (2H, s), 6.10 (1H, d), 6.40 (1H, dd), 7.36 (1H, t), 7.51 (1H, d), 7.66 (1H, d), 8.57 (1H, d)	—
24		a	65	163	222 (690), 281 (93), 290 (78)	1.01 (3H, d), 1.21 (3H, d), 6.10 (1H, d), 6.40 (1H, dd), 6.8~7.7 (8H, m), 8.56 (1H, d), 10.65 (1H, d)	—
25		a	71	251	251 (470)	0.86 (3H, t), 1.05 (3H, d), 6.1 (1H, d), 6.4 (1H, dd), 6.96 (2H, d), 7.36 (1H, t), 7.83 (2H, d), 7.96 (1H, d), 8.57 (1H, d)	575 (M^++1)[c]

[a] Synthetic routes as shown in Scheme 2, [b] yield based on **3** or **5**, [c] determined by SI-MS.

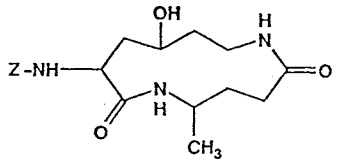
Table 2. Physico-chemical properties of glidobactin derivatives **26**~**35**.



Compound	Y	Route[a]	Yield[b] (%)	MP (°C)	UV λ_{max} nm ($E^{1\%}_{1cm}$)	Key signals in ^{1}H NMR (δ in ppm; 80 MHz, DMSO-d_6)	EI-MS (m/z)
26	(D)-Thr	b	5	203	259 (470)	0.86 (3H, t), 1.00 (3H, d), 6.20 (5H, m), 6.95 (1H, m), 7.35 (1H, t), 7.80 (2H, br d), 8.60 (1H, d)	521 (M^++1)[c]
27	(L)-Allo-Thr	b	15	180	262 (550)	0.86 (3H, t), 6.20 (5H, m), 7.00 (1H, m), 7.36 (1H, t), 8.13 (1H, d), 8.61 (2H, m)	279 (FA→Thr)[c]
28	(L)-NH-CH(C_2H_5)CO	b	35	254	260 (440)	0.86 (3H, t), 0.89 (3H, t), 6.20 (5H, m), 7.00 (1H, m), 7.35 (1H, t), 7.85 (1H, d), 7.97 (1H, d), 8.54 (1H, d)	504 (M^+)
29	(L)-Ser	b	17	206	262 (480)	0.86 (3H, t), 6.20 (5H, m), 7.00 (1H, m), 7.35 (1H, t), 7.75 (1H, d), 8.00 (1H, d), 8.56 (1H, d)	529 (M^++Na)[c]
30	(L)-Thr-(L)-Thr	a	22	204	265 (490)	0.86 (3H, t), 1.00 (6H, br d), 6.20 (5H, m), 7.00 (1H, m), 7.40 (2H, m), 7.60 (2H, m), 8.50 (1H, m)	586 (M^++1−2H_2O)[c]
31	(L)-Lys-N$^{\omega}$-Cbz	b	41	182	262 (470)	0.86 (3H, t), 4.98 (2H, s), 6.20 (5H, m), 6.90 (1H, m), 7.14 (1H, br s), 7.30 (6H, m), 7.97 (2H, br d), 8.60 (1H, m)	—
32	(L)-Lys	b	44[d]	226	259 (460)	0.86 (3H, t), 6.20 (5H, m), 7.00 (1H, m), 8.00 (2H, m), 8.60 (1H, m)	—
33	(L)-Glu-CH_3	b	44	215	262 (480)	0.86 (3H, t), 3.56 (3H, s), 6.20 (5H, m), 7.00 (1H, m), 7.35 (1H, t), 8.05 (2H, m), 8.55 (1H, d)	563 (M^++1)[c]
34	(L)-Glu	b	40[d]	208	259 (440)	0.86 (3H, t), 6.15 (5H, m), 8.00 (2H, m)	549 (M^++1)[c]
35	None	b	47	205	261 (670)	0.86 (3H, t), 6.20 (5H, m), 7.00 (1H, m), 7.36 (1H, t), 8.10 (1H, d), 8.62 (1H, d)	419 (M^+)

[a~c] See footnote in Table 1, [d] yield of the final deblocking reaction.

Table 3. Physico-chemical properties of glidobactin derivatives **36** and **37**.



Compound	Z	Route[a]	Yield[b] (%)	MP (°C)	UV λ_{max} nm ($E^{1\%}_{1cm}$)	Key signals in ^{1}H NMR (δ in ppm; 80 MHz, DMSO-d_6)	EI-MS (*m/z*)
36	H			210	End	1.05 (3H, d), 7.06 (1H, d), 7.70 (1H, t)	245 (M^+)
37	$CH_3(CH_2)_6(CH{=}CH)_2CONHCH(CH_3)CHOHCO-$ (E)	a	23	257	260 (530)	0.86 (3H, t), 1.02 (3H, d), 1.06 (3H, d), 6.12 (3H, m), 6.90 (1H, m), 7.30 (1H, d), 7.70 (3H, m)	522 (M^+)

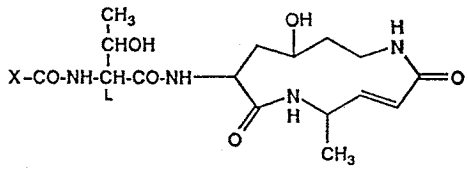
[a,b] See footnote in Table 1.

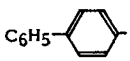
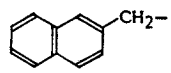
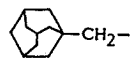
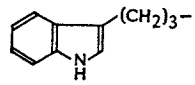
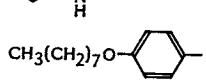
Results and Discussion

Thirty two derivatives prepared as above were evaluated for antifungal activity against *Candida albicans* A9540 (Ca-4) and *Saccharomyces cerevisiae* SC-1-109 (Sc-7), cytotoxicity against B16 melanoma cells and *in vivo* antitumor activity against P388 leukemia in mice. The results are summarized in Tables 4 and 5 comparatively with those of glidobactins A (**1a**) and C (**1c**).

The saturated fatty acid analogs exhibited stronger cytotoxicity and better *in vivo* antitumor activity

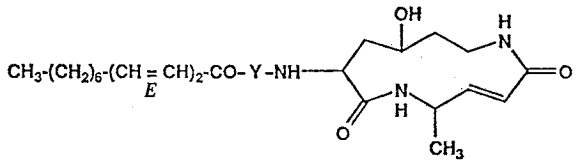
Table 4. Antitumor activity of glidobactin derivatives modified at the fatty acid.



Compound	X	Assay[a] (mm at 0.5 mg/ml)		MED[b] (μg/ml)	Anti-P388 activity[c] (% T/C of MST)			
		Ca-4	Sc-7	B16	10[d]	3	1	0.3
6	CH_3–	–	–	>250	105	100	100	
7	$CH_3(CH_2)_4$–	–	–	31	119	105	105	
8	$CH_3(CH_2)_6$–	–	13[w]	2	124	124	105	
9	$CH_3(CH_2)_8$–	14	23[w]	0.5	64	127	123	
10	$CH_3(CH_2)_{10}$–	15	25[w]	0.25	155	145	130	
11	$CH_3(CH_2)_8CH{=}CH$– (*E*)	14	28[w]	1		118	132	118
12	$HO(CH_2)_{11}$–	–	3[w]	4	Toxic	123	105	
13	$CH_3(CH_2)_{12}$–	12	19[w]	0.5	55	132	127	
14	$CH_3(CH_2)_{14}$–	–	12[w]	2	138	119	114	
15	$CH_3(CH_2)_{10}(CH{=}CH)_2$– (*E*)	12	19[w]	2	95	114	109	
16	$CH_3(CH_2)_{13}CH(OH)$–	–	13[w]	2	132	132	118	
17	C_6H_5–	–	–	8	109	95	91	
18	$C_6H_5CH_2$–	–	+[w]	8	109	95	95	
19	$C_6H_5CH{=}CC_6H_5$–	+[w]	25[w]	2.5	110	100	100	
20		–	16[w]	2.5	135	120	105	
21	$(C_6H_5)_2CH$–	–	+[w]	10	120	100	90	
22		–	+	2.5	110	100	95	
23		–	+	10	100	100	95	
24		–	–	10	115	100	95	
25		22	34[w]	0.4		Toxic	150	125
1a	$CH_3(CH_2)_6(CH{=}CH)_2$– (*E*)	23	36[w]	0.4		Toxic	145	130
1c	$CH_3(CH_2)_8(CH{=}CH)_2$– (*E*)	24	37[w]	1.6	57	129	124	

[a] Paper-disk assay against Ca-4 (*Candida albicans* A9540) and Sc-7 (*Saccharomyces cerevisiae* SC-1-109).
[b] Minimum effective dose to kill 90% of B16 melanoma cells[4].
[c] T/C values ≥125% are considered significant antitumor activity.
[d] Dose in mg/kg/day, qd 1→3, ip.
[w] Hazy inhibition zone.

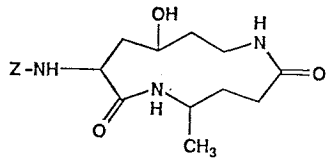
Table 5. Antitumor activity of glidobactin derivatives modified at the L-threonine moiety.



Compound	Y	Assay[a] (mm at 0.5 mg/ml)		MED[b] (μg/ml)	Anti-P388 activity[c] (% T/C of MST)			
		Ca-4	Sc-7	B16	10[d]	3	1	0.3
26	(D)-Thr	+	24[w]	1.6		127	118	109
27	(L)-Allo-Thr	14	25[w]	2		123	109	109
28	(L)-NHCH(C_2H_5)CO	+	22[w]	0.4	Toxic	138	119	
29	(L)-Ser	+	26[w]	1.6		91	118	114
30	(L)-Thr-(L)-Thr	−	19[w]	4	127	118	109	
31	(L)-Lys-N^{ω}-Cbz	−	18[w]	1	127	114	100	
32	(L)-Lys	−	14[w]	31		100	110	105
33	(L)-Glu-CH_3	−	+[w]	0.25	Toxic	132	123	
34	(L)-Glu	−	−	2		105	95	95
35	None	−	13[w]	4	115	110	110	

[a~d] See footnote in Table 4. [w] Hazy inhibition zone.

Table 6. Antitumor activity of glidobactin derivatives with the hydrogenated cyclic amine nucleus.



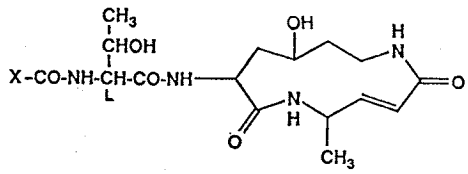
Compound	Z	Assay[a] (mm at 0.5 mg/ml)		MED[b] (μg/ml)	Anti-P388 activity[c] (% T/C of MST)		
		Ca-4	Sc-7	B16	10[d]	3	1
36	H	−	−	>250	105	100	100
37	$CH_3(CH_2)_6(CH{=}CH)_2CONH{-}\underset{L}{\underset{\vert}{\overset{\overset{CH_3}{\vert}}{CH}}}{-}CO-$	−	−	250	105	110	105
38[2]	$CH_3(CH_2)_{10}CONH{-}\underset{L}{\underset{\vert}{\overset{\overset{CH_3}{\vert}}{CH}}}{-}CO-$	−	−	250	105	95	100

[a~d] See footnote in Table 4.

than the corresponding α,β- or $\alpha,\beta,\gamma,\delta$-unsaturated fatty acid analogs (**10** vs. **11** and **1a**; **13** vs. **1c**; **14** vs. **15**). However, the antifungal activity of those saturated fatty acid derivatives was lower than that of the unsaturated counterparts.

Among the derivatives with a modified fatty acid moiety (Table 4), the saturated fatty acid analogs with short chain length are nearly inactive against fungi and tumor cells. The activity of the derivatives increases with the increase of chain length, reaching the maximum at C_{12}-acid (**10**) and then decreases with further increase of the chain length.

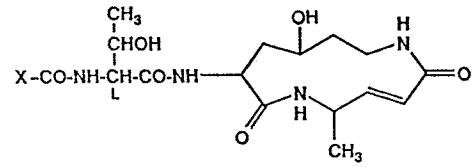
Table 7. Anti-P388 activity and acute toxicity of selected compounds.



Compound	X	Anti-P388 activity[a] (% T/C of MST)							LD_{50} (mg/kg)
		4[b]	2	1	0.5	0.25	0.13	0.063	
Glido-bactin A	$CH_3(CH_2)_6(CH{=}CH)_2$– (E)		Toxic	164	159	136	123	118	8.1
9	$CH_3(CH_2)_8$–	155	145	127	123	123	109		50
10	$CH_3(CH_2)_{10}$–	173	159	150	145	132	127		27
13	$CH_3(CH_2)_{12}$–	159	150	150	132	132	118		>25
25	$CH_3(CH_2)_7O$–C$_6$H$_4$–			Toxic	155	150	140	120	—

[a] T/C values ≥125% are considered significant antitumor activity.
[b] Dose in mg/kg/day, qd 1→9, ip.

Table 8. Anti-B16 melanoma activity of selected compounds.



Compound	X	Anti-B16 melanoma (ip-ip) activity[a] (% T/C of MST)					
		12[b]	8	4	2	1	0.5
Glidobactin A	$CH_3(CH_2)_6(CH{=}CH)_2$–				Toxic	116	113
10	$CH_3(CH_2)_{10}$–		Toxic	155	139	121	113
13	$CH_3(CH_2)_{12}$–	Toxic	153	145	124	116	113

[a] T/C values ≥125% are considered signifcant anti-B16 melanoma activity.
[b] See footnote in Table 7.

Derivatives having aromatic or alicyclic ring(s) in the side chain showed very weak or no activity in these tests except the *p-n*-octyloxybenzoyl derivative (**25**) which was comparable to **1a**.

It is interesting that introduction of a hydroxyl group at the α-position of C_{16}-acid (**16**) retains the biological activity while the presence of a hydroxyl group at ω-position of C_{12}-acid (**12**) decreases the activity.

Replacement of the L-threonine of **1a** with other amino acids (Table 5) mostly retains cytotoxicity and *in vivo* antitumor activity indicating that the hydroxyl group of L-threonine is not crucial for antitumor activity of the antibiotic. However, none of these amino acid analogs including D-threonine showed better activity than **1a**. Thus, L-threonine might be the best amino acid for antitumor activity in this series. The basic (L-lysyl, **32**) and acidic (L-glutamyl, **34**) amino acid derivatives, were completely inactive against P388 leukemia although their charge-protected derivatives (**31** and **33**) exhibited some antitumor effect. Three derivatives with the saturated cyclic amine nucleus (**36**, **37** and **38**) did not show antitumor activity (Table 6), indicating that the double bond in the cyclic amine moiety is essential for antitumor activity.

As shown in Table 7, the four most active derivatives (**9**, **10**, **13** and **25**) were further evaluated against P388 leukemia by a longer dosing schedule and for toxicity in mice. Compounds **10** and **13** showed a considerable reduction in toxicity without loss of antitumor activity resulting in higher chemotherapeutic indices than that of glidobactin A. Glidobactin A has no *in vivo* activity against B16 melanoma, while compounds **10** and **13** displayed significant prolongation of life span of mic inoculated with the tumor (Table 8).

Experimental

MP's were determined with a Yanagimoto micro hot-stage apparatus and not corrected. UV spectra were recorded on a Jasco UVIDEC-610 in MeOH solution and NMR spectra on a Varian FT-80A spectrometer. The ordinary mass spectra were obtained with a Hitachi RMU-6MG mass spectrometer modified with an in-beam electron impact system and secondary ion mass spectra (SI-MS) on a Hitachi M-80B (Xenon, 8 KeV). TLC was performed on precoated silica gel plates (Kieselgel $60F_{254}$, Merck, layer thickness 0.25 mm). Merck Kieselgel 60, and Merck Kieselgel 60 silanised were used for column chromatography.

Acetylglidobactamide (**6**)

To a stirred solution of **5** (8 mg, 0.023 mM) in 1 ml of MeOH was added acetic anhydride (10 mg) and the mixture was stirred for 2 hours at room temperature. The resulting solution was evaporated *in vacuo* to a residue which was taken up in MeOH (0.5 ml) and applied to a column of Sephadex LH-20 (10 ml). The column was developed with MeOH and the eluate examined by TLC (SiO_2, BuOH - AcOH - H_2O, 3 : 1 : 1, iodine detection). The fractions containing the major product were pooled, evaporated and lyophilized to give white amorphous solid of **6** (9.2 mg, 98% yield based on **5**). Its physico-chemical data are shown in Table 1.

Dodecanoylglidobactamide (**10**)

A mixture of **5** (21 mg, 0.06 mM) and dodecanoic anhydride (24 mg, 0.06 mM) in 1 ml of DMF was stirred overnight at room temperature. The mixture was diluted with 6 ml of MeOH and 4 ml of water and the solution applied to a column of reversed phase silica gel (40 ml). Upon elution with 70% MeOH, the appropriate fractions were pooled, evaporated and lyophilized to afford white amorphous solid of **10** (28 mg, 89%).

Compounds **9** and **13** were prepared by a similar procedure.

(*E*)-2-Dodecenoylglidobactamide (**11**)

A mixture of (*E*)-2-dodecenoic acid (13 mg, 0.064 mM), *N*,*N'*-dicyclohexylcarbodiimide (DCC, 10 mg, 0.064 mM) and 1-hydroxy-1,2,3-benzotriazole monohydrate (HOBT, 13 mg, 0.064 mM) in 2 ml of DMF was stirred for 2 hours at room temperature. Compound **5** (21 mg, 0.06 mM) was added to the solution and the mixture stirred overnight. After dilution with 60% aqueous MeOH (5 ml), the reaction mixture was chromatographed on reversed phase silica gel (30 ml) with 70% MeOH elution. The appropriate fractions were pooled, concentrated *in vacuo* and lyophilized to give compound **11** as white solid (25 mg, 83%).

Compounds **12**, **15**, **16**, **19**, **20**, **21**, **22**, **23**, **24**, **25**, **30**, **35** and **37** were synthesized by analogous procedures using appropriate acids and amines.

Phenylacetylglidobactamide (**18**)

To a stirred solution of **5** (21 mg, 0.06 mM) in 1 ml of DMF were added *N*,*O*-bis-(trimethylsilyl)-acetamide (0.03 ml) and phenylacetyl chloride (9.3 mg, 0.06 mM) at 5°C. After stirring for 3 hours at room temperature, the reaction mixture was diluted with 1 ml of water and applied on a column of reversed phase silica gel (30 ml). Elution with 30% aqueous MeOH yielded **18** as white amorphous solid (12.7 mg, 46%).

A similar acylation of **5** with benzoyl chloride gave compound **17**.

2-[(*E,E*)-2,4-Dodecadienoylamino]butyrylglidobactamide (**28**)

A mixture of (*E,E*)-2,4-dodecadienoic acid (**4**, 50 mg, 0.26 mM), DCC (58 mg, 0.28 mM) and HOBT (43 mg, 0.28 mM) in THF (5 ml) was stirred for 1 hour at room temperature. The mixture was filtered and the filtrate was added to a solution of L-2-aminobutyric acid (52 mg, 0.5 mM) and triethylamine (0.13 ml) in 50% aqueous THF (2 ml) under vigorous stirring. After being stirred an additional 4 hours, the reaction mixture was concentrated to 1 ml under reduced pressure and then diluted with 5 ml of water. The solution was washed with EtOAc (5 ml), acidified to pH 2.0 and extracted with EtOAc (5 ml). Evaporation of the extract gave a pale yellow solid of 2-[(*E,E*)-2,4-dodecadienoylamino]butyric acid (64 mg, 90%). The acid (28 mg, 0.1 mM) was dissolved in 5 ml of DMF solution containing DCC (21 mg, 0.1 mM) and HOBT (15 mg, 0.1 mM) and the mixture stirred for 1 hour. Compound **3** (24 mg, 0.1 mM) was then added to the solution and stirring continued overnight at room temperature. Concentration of the solution *in vacuo* yielded an oily residue which was loaded on a column of revesed phase silica gel (40 ml). Upon elution with 80% aqueous MeOH, the fractions containing the reaction product were pooled, concentrated and freeze-dried to give white solid of **28** (17.6 mg, 35%).

Preparation of compounds **7**, **8**, **14**, **26**, **27**, **29**, **31** and **33** followed a similar process as described above using appropriate fatty acids and amino acids.

(*E,E*)-2,4-Dodecadienoyl-L-lysylglidobactamide (**32**)

To a chilled solution of 20% HBr in AcOH (0.2 ml) was added **31** (16 mg, 0.023 mM) and the mixture was stirred at 5°C for 25 minutes. Twenty ml of ether was added to the solution to precipitate the product which was collected by filtration and dried *in vacuo* to yield 10 mg of yellow powder. This was chromatographed on silica gel (5 g) with BuOH - AcOH - H_2O (9 : 1 : 1) elution.

Evaporation of the appropriate fractions afforded semi-pure solid of **32** which was further purified by Sephadex LH-20 (20 ml) chromatography. Development of the column with MeOH yielded a homogeneous white solid of **32** (3.5 mg, 44%).

(*E,E*)-2,4-Dodecadienoyl-L-glutamylglidobactamide (**34**)

A suspension of **33** (10 mg, 0.018 mM) in 1 ml of MeOH containing KOH (10 mg) was stirred at 50°C for 2 hours. The resulting clear solution was diluted with water (2 ml), acidified to pH 2.0 and extracted with BuOH (4 ml). After being washed with water (1 ml), the extract was evaporated under reduced pressure to a residue which was chromatographed on Sephadex LH-20 (10 ml) with MeOH elution. Evaporation of the appropriate fractions afforded **34** (4 mg, 40%) as white amorphous powder.

Dihydroglidobamine (**36**)

Glidobamine (3.9 mg) in 50% aqueous MeOH (1 ml) was hydrogenated at 2.9 kg/cm^2 over 10% palladium on charcoal 1 mg in a Parr apparatus under shaking for 5 hours. After the catalyst was removed by filtration, the filtrate was concentrated and lyophilized to give 9 mg (98%) of **36**.

Its physico-chemical data are shown in Table 3.

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