Melophlins C−O, Thirteen Novel Tetramic Acids from the Marine Sponge *Melophlus sarassinorum*

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Received June 14, 2002

Thirteen new metabolites, melophlins C−O (**1**−**13**), were identified from the marine sponge *Melophlus sarassinorum*. Compounds **1**−**13** represent tetramic acid derivatives that differ with regard to the nature of their alkyl side chains. The structures of the new compounds were elucidated on the basis of comprehensive spectral analysis (^{1}H, ^{13}C, ^{1}H−^{1}H COSY, HMQC, and HMBC NMR, as well as low- and high-resolution ESIMS and EIMS). The absolute configurations of **1**, **8**, **10**, **11**, **12**, and **13** were determined by ESI LC/MS analysis of chiral derivatives obtained upon oxidation and hydrolysis of the respective parent compounds. Melophlin C (**1**) displayed pronounced antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*, together with antifungal activity against *Candida albicans*.

About one-third of all marine natural products known so far have been isolated from sponges, making them currently the most popular source of novel compounds.[1] Many of the chemically diverse marine sponge-derived secondary metabolites have pharmacological properties that render them interesting candidates for drug discovery.[2]

For sponges of the genus *Melophlus*, so far only two reports exist with regard to their secondary metabolites. Four triterpenoid saponins were isolated from a Guam sponge *Melophlus isis*,[3] whereas recently Kobayashi and co-workers investigated the Indonesian sponge *Melophlus sarassinorum* and reported two tetramic acid derivatives with a long alkyl side chain, namely, melophlins A and B. These compounds were reported to reverse the phenotype of *ras*-transformed cells.[4] In our continuing search for biologically active compounds from Indo-Pacific marine invertebrates,[5] the extract of the sponge *Melophlus sarassinorum*, collected near Makassar (Indonesia), showed moderate activity against larvae of the polyphageous pest insect *Spodoptera littoralis*. Bioassay-guided isolation afforded 13 new melophlin derivatives [melophlins C−O (**1**−**13**)]. The structures, including stereochemistry, were established on the basis of NMR and mass spectrometric data as well as by ESI LC/MS analysis.

Results and Discussion

The MeOH extract of the marine sponge *Melophlus sarrasinorum* showed moderate activity against larvae of the polyphageous pest insect *Spodoptera littoralis*. The EtOAc-soluble fraction obtained from the MeOH extract was found to have moderate growth-inhibition and insecticidal activities on *S. littoralis*. Subsequently, this fraction was subjected to repeated chromatographic steps, yielding 13 novel tetramic acids, melophlins C−O (**1**−**13**) (Scheme 1), as well as one known tetramic acid, melophlin A.[4]

Melophlin C (**1**), obtained as a yellow oil, showed a molecular ion peak in the low-resolution EIMS at *m*/*z* 323 $[M]^+$, and in the low-resolution ESIMS at *m*/*z* 324 $[M + H]^+$ as well as at *m*/*z* 322 $[M - H]^-$. In conjunction with 1D NMR data (Table 1), the molecular formula was deduced to be $C_{19}H_{33}NO_3$, which was subsequently confirmed by high-resolution ESIMS. The UV spectrum of **1** showed absorption maxima at 248 and 286 nm, suggesting the presence of a tetramic acid moiety.[6,7] Overall, the UV spectrum as well as the ^{1}H and ^{13}C NMR spectra of **1** were very reminiscent of melophlin B.[4] The ^{1}H NMR spectrum showed all signals at high field, disclosing the presence of one *N*-methyl singlet at δ 2.97, a long alkyl chain (δ 0.87, 3H, t, J = 6.9 Hz; δ 1.20−1.41, approximately 14H, m), and two aliphatic methyl doublets at δ 1.34 (J = 6.9 Hz) and 0.83 (J = 6.6 Hz), respectively. Furthermore, three multiplets at δ 2.81 (2H), 1.64 (2H), and 1.12 (2H) were observed, while the most downfield quartet at 3.68 (1H, J = 6.9 Hz) was attributed to a methine group which was adjacent to a heteroatom and carried a methyl substituent. The ^{13}C NMR and DEPT spectra revealed one *N*-methyl group (δ 26.2), three aliphatic methyl groups (δ 19.6, 14.8, and 14.1), and two methine carbons at δ 62.7 and 32.6, the former connected to a heteroatom. Furthermore, nine methylene signals were revealed at high field (δ 22.7−37.0). Four signals (δ 194.7, 187.9, 172.8, 100.5 for C-4, C-6, C-2, and C-3, respectively) were observed at low field which were almost identical to the respective signals reported for the tetramic acid ring system present in melophlin B.[4] The occurrence of the *N*-methyl group was indicated by its chemical shifts (δ_H 2.97, δ_C 26.2).[6]

Detailed interpretation of the one- and two-dimensional NMR spectra resulted in the elucidation of the structure of **1** as depicted. Thus, it was concluded that **1** was an

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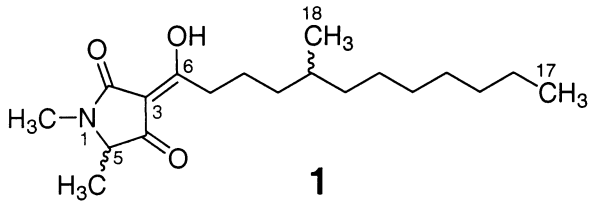
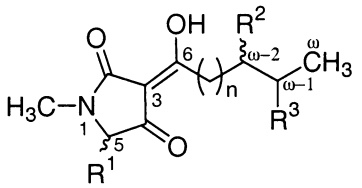
10.1021/np0202778 CCC: $25.00
Published on Web 12/07/2002

Scheme 1



1



2-13

melophlin	R^1	R^2	R^3	n
D (**2**)	H	H	H	11
E (**3**)	H	H	CH_3	11
F (**4**)	H	CH_3	H	11
G (**5**)	H	H	H	10
H (**6**)	H	H	CH_3	10
I (**7**)	H	CH_3	H	10
J (**8**)	CH_3	H	H	10
K (**9**)	H	H	CH_3	9
L (**10**)	CH_3	H	H	9
M (**11**)	CH_3	H	H	8
N (**12**)	CH_3	H	CH_3	8
O (**13**)	CH_3	CH_3	H	8

Table 1. NMR Spectral Data of Melophlin C (**1**)[a]

	δ_H (*J* Hz)	δ_C	HMBC
1			
2		172.8 s	
3		100.5 s	
4		194.7 s	
5	3.68 q (1H, 6.9)	62.7 d	2, 4, 5-CH_3
6		187.9 s	
7	2.81 m	32.8 t	3, 6, 8, 9
8	1.64 m	23.6 t	6, 9, 10
9	1.20−1.41 m	36.6 t	
10	1.20−1.41 m	32.6 d	
11	1.12 m	37.0 t	9, 13
12	1.20−1.41 m	26.3 t	
13	1.20−1.41 m	27.0 t	
14	1.20−1.41 m	31.9 t	
15	1.20−1.41 m	29.9 t	
16	1.20−1.41 m	22.7 t	
17	0.87 t (6.9)	14.1 q	15, 16
18	0.83 d (6.9)	19.6 q	9, 10, 11
5-CH_3	1.34 d (6.9)	14.8 q	4, 5
NCH_3	2.97 s	26.2 q	2, 5

[a] Recorded in $CDCl_3$.

isomer of melophlin B,[4] differing only in the position of the methyl substituent in the alkyl side chain. In the HMBC spectrum, the correlations from the *N*-methyl protons to C-2 and C-5, as well as from H-5 to C-2 and C-4 (see Table 1 and Figure 1), confirmed the tetramic acid moiety. The correlations from the methyl protons (5-CH_3) to C-5 (and vice versa) and C-4 indicated the position of the methyl substituent at C-5, which was also evident by the observation of the respective spin system in the $^1H-^1H$ COSY spectrum. This partial structure was also in agreement with ^1H and ^{13}C NMR data reported for tetramic acid derivatives in the literature.[4,6−12] The $^1H-^1H$ COSY spectrum also provided evidence of a second spin system, consisting of a long alkyl chain with two methyl groups. The ^{13}C chemical shifts (δ 14.1, C-17; 22.7, C-16; 29.9, C-15) indicated that the chain was terminated by a regular *n*-alkyl moiety, which was confirmed by HMBC correlations from H_3-17 to C-15 and C-16 (Table 1). Thus, the branching of the second methyl group (δ 19.6, C-18) had to be present in the interior of the alkyl chain. Its position at C-10 was deduced by the key ^3J correlation from H_2-8 to C-10 and by correlations from H_3-18 to C-10 and C-9, respectively, in the HMBC spectrum (see Table 1 and Figure 1). Furthermore, the position of the methyl substituent is also supported by the pronounced upfield shifts suffered by carbons 8 (δ 23.6) and 12 (δ 26.3), respectively, due to the γ-gauche effect. The assignments in the highly crowded aliphatic area of both the ^1H and the ^{13}C spectra were aided by HMBC correlations from H_2-7 to both C-3 and C-9 and careful interpretation of the COSY spectrum.

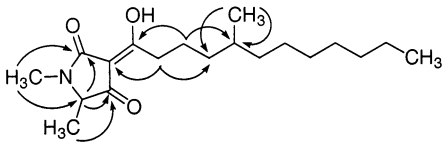


Figure 1. Selected HMBC correlations for melophlin C (**1**).

Similar to the observation reported for melophlins A and B,[4] the ^1H and ^{13}C NMR spectra of **1** (data not shown) revealed that **1** existed as a 9:1 mixture of two tautomers, designated as exo A and exo B form of the tetramic acid moiety.[13−15] Furthermore, the ^{13}C NMR spectrum of **1** clearly showed double sets of signals for the chiral center at C-10 and the adjacent carbons (C-10, δ 32.6, 32.5; C-9, δ 36.6, 36.5; C-11, δ 37.0, 36.9; and C-18, δ 19.6, 19.5), which were in a similar ratio of approximately 10:6. Thus, it was concluded that compound **1** was an inseparable mixture of two diastereomers differing in the configuration at C-10.

The absolute stereochemistry of the chiral center at C-5 was determined using the advanced Marfey's method, which involved ESI LC/MS analysis of a chiral derivative of **1** obtained upon derivatization.[4,16−19] Specifically, compound **1** was oxidized with $NaIO_4$ and $KMnO_4$, followed by hydrolysis with 2 N aqueous HCl, yielding enantiomers of *N*-methylalanine, which were subsequently treated with *N*-(5-fluoro-2,4-dinitrophenyl-5)-L-leucinamide (L-FDLA). Two peaks at a ratio of approximately 2:1 (*N*-methyl-L-alanine-FDLA:*N*-methyl-D-alanine-FDLA) were found by ESI LC/MS. Both the retention times and the online ESI mass spectra of these peaks were essentially identical to the corresponding derivatives obtained from commercially available *N*-methyl-L-alanine and *N*-methyl-DL-alanine. Thus, this finding suggests that melophlin C (**1**) was isolated as a 2:1 mixture of the 5(*S*)- and 5(*R*)-enantiomers. This result was somewhat surprising, as similar observations were not reported in the original communication when melophlin B was subjected to an identical analytical procedure.[4] Even when milder conditions for the derivatization reaction were applied, both enantiomers were detected in a similar ratio.[20] Attempts to directly detect

Table 2. ^{1}H and ^{13}C NMR Data of Melophlins D (**2**), E (**3**), and F (**4**)[a]

	2		**3**		**4**	
	δ_H (*J* Hz)	δ_C	δ_H (*J* Hz)	δ_C	δ_H (*J* Hz)	δ_C
1						
2		173.5 s		173.5 s		173.6 s
3		101.6 s		101.6 s		101.6 s
4		191.3 s		191.3 s		191.3 s
5	3.71 s (2H)	57.7 t	3.74 s (2H)	57.7 t	3.72 s (2H)	57.7 t
6		187.6 s		187.6 s		187.6 s
7	2.81 t (7.6)	32.6 t	2.84 t (7.6)	32.6 t	2.82 t (7.6)	32.6 t
8	1.64 m	26.0 t	1.67 m	26.0 t	1.65 m	26.0 t
9-[ω-4]	1.20−1.39 (m)	29.2−29.7 (8× t)	1.22−1.42 (m)	29.2−29.9 (8× t)	1.20−1.40 (m)	27.1−30.0 (8× t)
[ω-3]	1.20−1.39 (m)	31.9 t	1.22−1.42 (m)	27.4 t	1.20−1.40 (m)	36.6 t
[ω-2]	1.20−1.39 (m)	29.2−29.7 (1× t)	1.22−1.42 (m)	39.0 t	1.15 (m)	34.4 d
[ω-1]	1.29 m	22.7 t	1.53 m (6.6)	28.0 d	1.20−1.40 (m)	29.7 t
ω	0.87 t (6.6)	14.1 q	0.88 2d (6H, 6.6)	22.6 (2× q)	0.85 t (7.3)	11.4 q
[ω-2]-CH_3					0.83 d (6.3)	19.2 q
NCH_3	3.01 s	28.4 q	3.04 s	28.4 q	3.02 s	28.4 q

[a] Recorded in $CDCl_3$.

both enantiomers of melophlin C (**1**) by HPLC using chiral stationary phases without prior derivatization proved unsuccessful. Taking the above-mentioned observation of the suspected C-10 diastereomers into account, we concluded that melophlin C (**1**) was actually obtained as an inseparable mixture of all four possible stereoisomers at C-5 and C-10, together with the observation of tetramic acid exo A and exo B tautomers (see discussion above).

Melophlin D (**2**), also obtained as a yellow oil, showed a quasi-molecular ion peak in the positive and negative low-resolution ESIMS at *m*/*z* 338 $[M + H]^+$ and 336 $[M - H]^-$, respectively. Together with the consideration of ^{1}H and ^{13}C NMR data (Table 2) $C_{20}H_{35}NO_3$ was assigned as molecular formula, which was also confirmed by high-resolution ESIMS. The UV spectrum and the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of **2** showed close similarity to those of melophlin C (**1**) as well as to those reported for melophlin A.[4] Detailed comparison of the NMR data revealed that melophlin D (**2**) was devoid of both the methyl substituent at C-5 in the tetramic acid moiety and the branching methyl group in the alkyl side chain present in **1**. Also the ^{1}H NMR signal of H-5 which was observed as a quartet at δ 3.68 (1H) in **1** appeared as a singlet at δ 3.71 (2H) in **2**, while the DEPT spectrum revealed that the doublet due to C-5 (δ 62.7) in **1** was replaced by a triplet (δ 57.7) in **2**. Other proton and carbon signals of **2** showed slight chemical shift differences compared to **1** obviously caused by the missing substituent at C-5: NCH_3 δ_H from 2.97 to 3.01, and δ_C from 26.2 to 28.4; C-2 δ_C from 172.8 to 173.5, C-3 δ_C from 100.5 to 101.6, and C-4 δ_C from 194.7 to 191.3. All these data were in excellent agreement with the respective differences reported for melophlin A compared to melophlin B,[4] which also differ by a methyl substituent at C-5. Thus, it was concluded that melophlin D (**2**) was identical to melophlin A with regard to the tetramic acid moiety, but was lacking one methylene group in the alkyl side chain compared to the latter (Scheme 1). The construction of the tetramic acid moiety in **2** was further confirmed by the HMBC correlations observed for melophlin I (**7**) (see below), of which the respective NMR signals of the heterocycle were virtually identical to those observed for **2**.

Once the structures of melophlin C and D (**1** and **2**) were determined, the structure elucidation of melophlins E−O (**3**−**13**) proceeded in a very straightforward manner. By comparison of the UV and 1D NMR data, it could be easily shown that all compounds contained the same tetramic acid core (either with or without the methyl substituent at C-5) attached to a long alkyl side chain termination (Scheme 1), namely, those with a linear *n*-alkyl side chain (**5**, **8**, **10**, and **11**), those with an *iso*-type methyl branched chain terminus (**3**, **6**, **9**, and **12**), and those with an *anteiso*-type methyl branched chain terminus (**4**, **7**, and **13**). The overall chain length of each individual compound was derived from the molecular formula, which was readily deduced from the low-resolution ESIMS or EIMS spectra, respectively. In all cases, the result was confirmed by high-resolution ESIMS or EIMS, respectively, similar to the procedure described above for melophlins C (**1**) and D (**2**) (see Experimental Section).

Melophlins G, J, L, and M (**5**, **8**, **10**, and **11**) contained a linear *n*-alkyl side chain similar to the one present in **2**, while melophlins E, H, K, and N (**3**, **6**, **9**, and **12**) displayed an *iso*-type methyl branching at the terminus of the alkyl chain. In the ^{13}C and DEPT spectra of the latter, characteristic signals appeared for the terminal carbon atoms, i.e., C_ω, δ 22.6 (2 × q); $C_{\omega-1}$, δ 27.9−28.0 (d); and $C_{\omega-2}$, δ 39.0 (t) (see Table 2 and Experimental Section). Finally, melophlins F, I, and O (**4**, **7**, and **13**) contained an *anteiso*-type methyl branched alkyl side chain. This type of termination was easily recognized in the ^{13}C spectrum through the pronounced upfield shift of the terminal carbon (δ 11.4, q) due to the γ-effect of the additional methyl group at $C_{\omega-2}$. Other diagnostic ^{13}C chemical shifts included $C_{\omega-1}$, δ 29.7 (t), $C_{\omega-2}$, δ 34.4 (d), $C_{\omega-3}$, δ 36.6 (t), and $C_{\omega-2}-CH_3$, δ 19.2 (q) (see Table 2 and Experimental Section). In the case of melophlin I (**7**), the *anteiso*-branching as well as the nature of the tetramic acid moiety was confirmed by HMBC (see Experimental Section).

The absolute stereochemistry at C-5 of melophlins J (**8**), L (**10**), M (**11**), N (**12**), and O (**13**) was also analyzed with the advanced Marfey's method by ESI LC/MS. Similar to the results discussed above for melophlin C (**1**), two peaks were observed in a ratio of approximately 2:1 in each case, except for **8**, which had a ratio of approximately 2:3. These results indicate that **8**, **10**, **11**, **12**, and **13** were isolated as a mixture of the respective 5(*S*)- and 5(*R*)-enantiomers. As with melophlin C (**1**), attempts to directly detect both enantiomers of melophlin M (**11**) by chiral chromatography without prior derivatization proved unsuccessful.

In the literature there have been numerous reports on tetramic acid glycosides from marine sponges, such as ancorinoside A, an inhibitor of starfish embryo blastulation from *Ancorina* sp.,[6] and ancorinosides B−D, inhibitors of type 1 matrix metalloproteinase (MT1-MMP) from *Penares sollasi*.[8] A common feature among sponge-derived tetramic acid derivatives is a long conjugated polyene side chain. For example, this structural feature is present in aurantosides A and B with cytotoxic activity from *Theonella* sp.,[9]

aurantoside C from *Homophymia conferta*,[10] aurantosides D−F with antifungal activity from *Siliquariaspongia japonica*,[11] and rubrosides A−H inducing intracellular vacuoles in 3Y1 rat fibroblasts and displaying cytotoxic and antifungal activities from *Siliquariaspongia japonica*.[12] Interestingly, similar structures have been reported from actinomycetes (α- and *β*-lipomycin from *Streptomyces aureofaciens*),[21] imperfect fungi (erythroskyrin from *Penicillium islandicum*),[22] and slime molds (physarubinic acid from *Physarum polycephalum*).[23] Structurally complex cyclic tetramic acid derivatives of mixed biogenetic origin displaying structural similarities to the known actinomycete-derived antibiotic ikarugamycin have also been described from sponges. Examples include discodermide, exhibiting antifungal and cytotoxic activities, from *Discodermia dissoluta*,[24] cylindramide from *Halichondria cylindrata*,[25] and the closely related geodin A from *Geodia* sp.[26] Two unusually substituted tetramic acid derivatives, ascosalipyrrolidinones A and B, were reported from the obligate marine fungus *Ascochyta salicorniae* isolated from the green alga *Ulva* sp.[27] The latter compounds were related to equisetin and phomasetin, obtained from the terrestrial fungi *Fusarium equiseti*, *F. heterosporum*, and *Phoma* sp., respectively.[28]

In the original report, melophlins A and B were reported to reverse the phenotype of H-*ras*-transformed NIH3T3 fibroblast, besides displaying moderate cytotoxicity against HL60 cells.[4] These results prompted us to investigate the antiproliferative properties of the compounds presented in this paper. In contrast to the previous report, we could not detect any significant cytotoxic activity of melophlins C (**1**), E (**3**), G (**5**), H (**6**), I (**7**), M (**11**), N (**12**), or O (**13**) against the HL-60 cell line. The said compounds also proved inactive against HELA and TF-1 cells, respectively. However, melophlin C (**1**) displayed pronounced antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus*, together with antifungal activity against *Candida albicans*.[29] In the same test systems, melophlins G (**5**) and I (**7**) were only moderately active.[29] When tested against larvae of the polyphageous pest insect *Spodoptera littoralis*, melophlins C (**1**), G (**5**), and I (**7**) displayed moderate activity. Furthermore, melophlins C (**1**), G (**5**), and I (**7**) were active against brine shrimp, *Artemia salina*, with IC_{50} values of 36.6, 48.8, and 52.6 *μ*g/mL, respectively.

Experimental Section

General Experimental Procedures. Optical rotation was recorded on a Perkin-Elmer Model 341 LC polarimeter. UV spectra were obtained in methanol using a Beckman Model 25 spectrophotometer. NMR spectral data were acquired on Bruker DRX-500 MHz and Bruker DRX-600 MHz NMR spectrometers. Low- and high-resolution ESIMS analyses were performed on a Micromass Qtof 2 mass spectrometer. Low- and high-resolution EIMS were measured with a Finnigan MAT 311A mass spectrometer at 70 eV. HPLC−UV analyses were conducted with a Dionex system coupled to a photodiode array detector using a 5 *μ*m Eurospher-100 C18 column (4 mm i.d. × 150 mm; Knauer, Berlin, Germany). Routine detection was at 283 nm. Semipreparative HPLC was performed on a Merck-Hitachi instrument (pump L-7100, detector L-7400) using a 7 *μ*m Eurospher-100 C18 column (8 mm i.d. × 300 mm; Knauer, Berlin, Germany). Chiral CC 30/4 HPLC columns (Nucleodex *β*-PM, Nucleodex *γ*-PM, and Nucleodex *β*-OH) were purchased from Macherey-Nagel, Düren, Germany. Column chromatography was performed on Sephadex LH-20 (Sigma-Aldrich, Steinheim, Germany), silica gel (0.040−0.063 mm; Merck, Darmstadt, Germany), or RP-18 Lobar columns (40−63 *μ*m, 25 mm i.d. × 310 mm, Merck, Darmstadt, Germany), and TLC analyses were carried out using aluminum sheet precoated silica gel 60 F_{254} (Merck, Darmstadt, Germany). Detection was by UV absorption at 254 nm. All solvents used were distilled prior to use. Spectral grade solvents were utilized for chromatographic analysis. Standard amino acids, *N*-methyl-alanine-OH, *N*-methyl-DL-alanine-OH, and reagents for derivatization were purchased from Bachem, Heidelberg, Germany. *N*-(5-Fluoro-2,4-dinitrophenyl-5)-L-leucinamide (advanced Marfey's reagent, L-FDLA) was purchased from TCI, Tokyo Kasei Kogyo, Japan.

Extraction and Isolation. The marine sponge *Melophlus sarassinorum* Thiele (order Astrophorida, family Ancorinidae) was collected in 1996 at Barang Lompo island close to Makassar (Sulawesi, Indonesia). It was a large bodied sponge with hard consistency, pale brown in color, with a warty-bumpy surface provided with numerous holes. The skeleton was made up of large smooth oxeas of variable size, length × width 622−1285 × 11−48 *μ*m, arranged confusedly or in poorly defined tracts in the white interior. At the surface, oxeas were arranged tangentially. Microscleres comprised spined microrhabds and centrotylote microxeas, with widely different sizes grading into each other, length × width 13−123 × 1−5 *μ*m, concentrated densely at the surface, occurring more dispersed in the interior, and oxyasters or occasionally tylasters with a small but pronounced center and 8−12 spined rays, diameter 12−26 *μ*m, occurring in the interior only. A voucher fragment of the sponge was deposited in the Zoological Museum, University of Amsterdam, under registration number ZMA POR. 16276.

The sponge (dry weight, 85 g) was repeatedly extracted with MeOH (1000 mL × 4) at room temperature for 5 h under stirring. The combined extracts were evaporated under reduced pressure and taken to dryness (10.5 g). The residue was partitioned between cyclohexane (300 mL × 3) and H_2O (300 mL), EtOAc (200 mL × 3) and H_2O (200 mL), and *n*-BuOH (200 mL × 3) and H_2O (200 mL), successively. The organic fractions were evaporated to give the cyclohexane-soluble portion (1.1 g), EtOAc-soluble portion (1.5 g), and *n*-BuOH-soluble portion (6.3 g), respectively. The EtOAc-soluble portion was subjected to column chromatography over Sephadex LH-20 with MeOH as mobile phase to yield five fractions. The main fraction, fraction 3, was subjected to column chromatography on silica gel 60 (CH_2Cl_2−MeOH, 80:20), and RP-HPLC with MeOH and H_2O (0.1% TFA) using the following gradient: 0−5 min, 75% MeOH; 10−70 min, 83% MeOH; 80−100 min, 87% MeOH, yielding melophlin C (**1**, 32.4 mg), melophlin D (**2**, 43.6 mg), melophlin E (**3**, 16.7 mg), melophlin F (**4**, 5.1 mg), melophlin G (**5**, 49.9 mg), melophlin H (**6**, 44.3 mg), melophlin I (**7**, 14.9 mg), melophlin J (**8**, 15.0 mg), melophlin K (**9**, 14.5 mg), melophlin L (**10**, 9.8 mg), melophlin M (**11**, 12.8 mg), an inseparable mixture of melophlins N and O (**12** and **13**, 47.3 mg), and the known melophlin A (45.7 mg).

Melophlin C (1): yellow oil (MeOH); $[\alpha]_D^{20}$ −5.8° (*c* 0.35, MeOH); UV (MeOH) λ_{max} (log ϵ) 248 (3.90), 286 (4.12) nm; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 323 $[M]^+$ (10), 182 (28), 169 (100), 154 (58); ESIMS pos *m/z* 324 $[M + H]^+$ (100); ESIMS neg *m/z* 322 $[M - H]^-$ (100); HRESIMS pos *m/z* 324.2502 $[M + H]^+$ (calcd for $C_{19}H_{34}NO_3$, 324.2538).

Melophlin D (2): yellow oil (MeOH); UV (MeOH) λ_{max} (log ϵ) 247 (3.74), 286 (3.93) nm; ^1H and ^{13}C NMR data, see Table 2; EIMS *m/z* 337 $[M]^+$ (2), 169 (5), 155 (10); ESIMS pos *m/z* 338 $[M + H]^+$ (100); ESIMS neg *m/z* 336 $[M - H]^-$ (100); HRESIMS pos *m/z* 338.2672 $[M + H]^+$ (calcd for $C_{20}H_{36}NO_3$, 338.2695).

Melophlin E (3): yellow oil (MeOH); UV (MeOH) λ_{max} (log ϵ) 246 (4.08), 286 (4.20) nm; ^1H and ^{13}C NMR data, see Table 2; EIMS *m/z* 351 $[M]^+$ (15), 168 (25), 155 (100), 140 (30); ESIMS pos *m/z* 352 $[M + H]^+$ (100); ESIMS neg *m/z* 350 $[M - H]^-$ (100); HRESIMS pos *m/z* 352.2833 $[M + H]^+$ (calcd for $C_{21}H_{38}NO_3$, 352.2851).

Melophlin F (4): yellow oil (MeOH); $[\alpha]_D^{20}$ −2.0° (*c* 0.35, MeOH); UV (MeOH) λ_{max} (log ϵ) 245 (3.06), 286 (3.15) nm; ^1H and ^{13}C NMR data, see Table 2; EIMS *m/z* 351 $[M]^+$ (8), 168

(15), 155 (45), 140 (15); ESIMS pos *m/z* 352 $[M + H]^+$ (100); HRESIMS pos *m/z* 352.2827 $[M + H]^+$ (calcd for $C_{21}H_{38}NO_3$, 352.2851).

Melophlin G (5): yellow oil (MeOH); UV (MeOH) λ_{max} (log ϵ) 247 (4.00), 285 (4.19) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.71 (2H, s, H_2-5), 3.01 (3H, s, NCH_3), 2.81 (2H, t, J = 7.6 Hz, H_2-7), 1.64 (2H, m, H_2-8), 1.20−1.38 (20H, m, H_2-9-$H_{2[\omega-1]}$), 0.87 (3H, t, J = 6.9 Hz, $H_{3[\omega]}$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 191.3 (s, C-4), 187.6 (s, C-6), 173.5 (s, C-2), 101.6 (s, C-3), 57.6 (t, C-5), 32.6 (t, C-7), 31.9 (t, $C_{\omega-3}$), 29.2−29.6 (8 × t, C-9-$C_{\omega-4}$, $C_{\omega-2}$), 28.4 (q, NCH_3), 26.0 (t, C-8), 22.7 (t, $C_{\omega-1}$), 14.1 (q, C_ω); EIMS *m/z* 323 $[M]^+$ (15), 168 (20), 155 (100), 140 (30); ESIMS pos *m/z* 324 $[M + H]^+$ (100); ESIMS neg *m/z* 322 $[M - H]^-$ (100); HREIMS *m/z* 323.2446 $[M]^+$ (calcd for $C_{19}H_{33}NO_3$, 323.2460).

Melophlin H (6): yellow oil (MeOH); UV (MeOH) λ_{max} (log ϵ) 247 (4.01), 285 (4.25) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.71 (2H, s, H_2-5), 3.01 (3H, s, NCH_3), 2.81 (2H, t, J = 7.6 Hz, H_2-7), 1.64 (2H, m, H_2-8), 1.50 (1H, m, J = 6.6 Hz, $H_{\omega-1}$), 1.20−1.39 (18H, m, H_2-9-$H_{2[\omega-2]}$), 0.86 (6H, 2 × d, J = 6.6 Hz, 2 × $H_{3[\omega]}$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 191.3 (s, C-4), 187.6 (s, C-6), 173.5 (s, C-2), 101.6 (s, C-3), 57.6 (t, C-5), 39.0 (t, $C_{\omega-2}$), 32.6 (t, C-7), 29.2−29.9 (7 × t, C-9-$C_{\omega-4}$), 28.4 (q, NCH_3), 27.9 (d, $C_{\omega-1}$), 27.4 (t, $C_{\omega-3}$), 26.0 (t, C-8), 22.6 (2 × q, 2 × C_ω); ESIMS pos *m/z* 338 $[M + H]^+$ (100); ESIMS neg *m/z* 336 $[M - H]^-$ (100); HRESIMS pos *m/z* 338.2650 $[M + H]^+$ (calcd for $C_{20}H_{36}NO_3$, 338.2695).

Melophlin I (7): yellow oil (MeOH); $[\alpha]_D^{20}$ −2.6° (*c* 0.34, MeOH); UV (MeOH) λ_{max} (log ϵ) 245 (3.27), 286 (3.47) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.71 (2H, s, H_2-5), 3.01 (3H, s, NCH_3), 2.81 (2H, t, J = 7.6 Hz, H_2-7), 1.65 (2H, m, H_2-8), 1.12−1.39 (19H, m, H_2-9-$H_{2[\omega-3]}$, $H_{\omega-2}$, $H_{2[\omega-1]}$), 0.85 (3H, t, J = 7.3 Hz, $H_{3[\omega]}$), 0.83 (3H, d, J = 6.3 Hz, [ω-2]-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 191.3 (s, C-4), 187.6 (s, C-6), 173.5 (s, C-2), 101.6 (s, C-3), 57.7 (t, C-5), 36.6 (t, $C_{\omega-3}$), 34.4 (d, $C_{\omega-2}$), 32.6 (t, C-7), 29.2−29.7 (6 × t, C-9-$C_{\omega-4}$), 29.7 (t, $C_{\omega-1}$), 27.1 (t, C-15), 28.4 (q, NCH_3), 26.0 (t, C-8), 19.2 (q, [ω-2]-CH_3), 11.4 (q, C_ω); HMBC C-2 (NCH_3, H_2-5), C-3 (H_2-7), C-4 (H_2-5), C-5 (NCH_3), C-6 (H_2-7, H_2-8), C-7 (H_2-8), C-8 (H_2-7), $C_{\omega-3}$ ([ω-2]-CH_3), $C_{\omega-2}$ ($H_{3[\omega]}$, [ω-2]-CH_3), $C_{\omega-1}$ ($H_{3[\omega]}$, [ω-2]-CH_3); EIMS *m/z* 337 $[M]^+$ (20), 168 (25), 155 (100), 140 (30); ESIMS pos *m/z* 338 $[M + H]^+$ (100); ESIMS neg *m/z* 336 $[M - H]^-$ (100); HREIMS *m/z* 337.2633 $[M]^+$ (calcd for $C_{20}H_{35}NO_3$, 337.2617).

Melophlin J (8): yellow oil (MeOH); $[\alpha]_D^{20}$ −2.6° (*c* 0.31, MeOH); UV (MeOH) λ_{max} (log ϵ) 246 (3.11), 286 (3.25) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.68 (1H, q, J = 6.9 Hz, H-5), 2.97 (3H, s, NCH_3), 2.82 (2H, m, J = 7.3 Hz, H_2-7), 1.64 (2H, m, H_2-8), 1.35 (3H, d, J = 6.9 Hz, 5-CH_3), 1.20−1.39 (20H, m, H_2-9-$H_{2[\omega-1]}$), 0.87 (3H, t, J = 6.9 Hz, $H_{3[\omega]}$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 194.7 (s, C-4), 187.9 (s, C-6), 172.8 (s, C-2), 100.5 (s, C-3), 62.7 (d, C-5), 32.6 (t, C-7), 31.9 (t, $C_{\omega-3}$), 29.2−29.7 (8 × t, C-9-$C_{\omega-4}$, $C_{\omega-2}$), 26.2 (q, NCH_3), 26.0 (t, C-8), 22.7 (t, $C_{\omega-1}$), 14.8 (q, 5-CH_3), 14.1 (q, C_ω); HMBC C-2 (NCH_3, H-5), C-3 (H_2-7), C-4 (H-5, 5-CH_3), C-5 (NCH_3, 5-CH_3), C-6 (H_2-7, H_2-8), C-8 (H_2-7), $C_{\omega-2}$ ($H_{3[\omega]}$), $C_{\omega-1}$ ($H_{3[\omega]}$), 5-CH_3 (H-5); EIMS *m/z* 337 $[M]^+$ (15), 182 (20), 169 (80), 154 (30); ESIMS pos *m/z* 338 $[M + H]^+$ (100); ESIMS neg *m/z* 336 $[M - H]^-$ (100); HRESIMS pos *m/z* 338.2714 $[M + H]^+$ (calcd for $C_{20}H_{36}NO_3$, 338.2695).

Melophlin K (9): yellow oil (MeOH); UV (MeOH) λ_{max} (log ϵ) 243 (3.04), 286 (3.09) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.72 (2H, s, H_2-5), 3.01 (3H, s, NCH_3), 2.82 (2H, t, J = 7.6 Hz, H_2-7), 1.65 (2H, m, H_2-8), 1.51 (1H, m, J = 6.6 Hz, $H_{\omega-1}$), 1.20−1.39 (16H, m, H_2-9-$H_{2[\omega-2]}$), 0.86 (6H, 2 × d, J = 6.6 Hz, 2 × $H_{3[\omega]}$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 191.3 (s, C-4), 187.6 (s, C-6), 173.5 (s, C-2), 101.6 (s, C-3), 57.7 (t, C-5), 39.0 (t, $C_{\omega-2}$), 32.6 (t, C-7), 29.2−29.9 (6 × t, C-9-$C_{\omega-4}$), 28.4 (q, NCH_3), 27.9 (d, $C_{\omega-1}$), 27.4 (t, $C_{\omega-3}$), 26.0 (t, C-8), 22.6 (2 × q, 2 × C_ω); EIMS *m/z* 323 $[M]^+$ (15), 182 (20), 169 (65), 155 (85); ESIMS pos *m/z* 324 $[M + H]^+$ (100); ESIMS neg *m/z* 322 $[M - H]^-$ (100); HRESIMS pos *m/z* 324.2521 $[M + H]^+$ (calcd for $C_{19}H_{34}NO_3$, 324.2538).

Melophlin L (10): yellow oil (MeOH); $[\alpha]_D^{20}$ −3.8° (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ϵ) 245 (3.00), 286 (3.03) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.68 (1H, q, J = 6.9 Hz, H-5), 2.97 (3H, s, NCH_3), 2.82 (2H, m, H_2-7), 1.64 (2H, m, H_2-8), 1.35 (3H, d, J = 6.9 Hz, 5-CH_3), 1.20−1.39 (18H, m, H_2-9-$H_{2[\omega-1]}$), 0.87 (3H, t, J = 6.9 Hz, $H_{3[\omega]}$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 194.7 (s, C-4), 188.0 (s, C-6), 172.8 (s, C-2), 100.5 (s, C-3), 62.7 (d, C-5), 32.6 (t, C-7), 31.9 (t, $C_{\omega-3}$), 29.2−29.7 (7 × t, C-9-$C_{\omega-4}$, $C_{\omega-2}$), 26.3 (q, NCH_3), 26.0 (t, C-8), 22.7 (t, $C_{\omega-1}$), 14.8 (q, 5-CH_3), 14.1 (q, C_ω); EIMS *m/z* 323 $[M]^+$ (10), 182 (28), 169 (100), 154 (40); ESIMS pos *m/z* 324 $[M + H]^+$ (100); ESIMS neg *m/z* 322 $[M - H]^-$ (100); HRESIMS pos *m/z* 324.2501 $[M + H]^+$ (calcd for $C_{19}H_{34}NO_3$, 324.2538).

Melophlin M (11): yellow oil (MeOH); $[\alpha]_D^{20}$ −5.0° (*c* 0.38, MeOH); UV (MeOH) λ_{max} (log ϵ) 248 (3.53), 286 (3.69) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.68 (1H, q, J = 6.9 Hz, H-5), 2.97 (3H, s, NCH_3), 2.82 (2H, m, H_2-7), 1.64 (2H, m, H_2-8), 1.35 (3H, d, J = 6.9 Hz, 5-CH_3), 1.20−1.42 (16H, m, H_2-9-$H_{2[\omega-1]}$), 0.87 (3H, t, J = 6.9 Hz, $H_{3[\omega]}$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 194.7 (s, C-4), 187.9 (s, C-6), 172.8 (s, C-2), 100.5 (s, C-3), 62.7 (d, C-5), 32.6 (t, C-7), 31.9 (t, $C_{\omega-3}$), 29.2−29.7 (6 × t, C-9-$C_{\omega-4}$, $C_{\omega-2}$), 26.2 (q, NCH_3), 26.0 (t, C-8), 22.7 (t, $C_{\omega-1}$), 14.8 (q, 5-CH_3), 14.1 (q, C_ω); EIMS *m/z* 309 $[M]^+$ (9), 182 (28), 169 (100), 154 (55); ESIMS pos *m/z* 310 $[M + H]^+$ (100); ESIMS neg *m/z* 308 $[M - H]^-$ (100); HRESIMS pos *m/z* 310.2340 $[M + H]^+$ (calcd for $C_{18}H_{32}NO_3$, 310.2382).

Melophlin N (12): yellow oil (MeOH); UV (MeOH) λ_{max} (log ϵ) 248 (3.79), 286 (3.99) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.68 (1H, q, J = 6.9 Hz, H-5), 2.96 (3H, s, NCH_3), 2.82 (2H, m, H_2-7), 1.64 (2H, m, H_2-8), 1.50 (1H, m, J = 6.6 Hz, $H_{\omega-1}$), 1.34 (3H, d, J = 6.9 Hz, 5-CH_3), 1.20−1.39 (14H, m, H_2-9-$H_{2[\omega-2]}$), 0.86 (6H, 2 × d, J = 6.6 Hz, 2 × $H_{3[\omega]}$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 194.7 (s, C-4), 187.9 (s, C-6), 172.8 (s, C-2), 100.5 (s, C-3), 62.7 (d, C-5), 39.0 (t, $C_{\omega-2}$), 32.6 (t, C-7), 29.2−29.9 (5 × t, C-9-$C_{\omega-4}$), 27.9 (d, $C_{\omega-1}$), 27.4 (t, $C_{\omega-3}$), 26.2 (q, NCH_3), 26.0 (t, C-8), 22.6 (2 × q, 2 × C_ω), 14.8 (q, 5-CH_3); EIMS *m/z* 323 $[M]^+$ (10), 182 (25), 169 (100), 154 (30); ESIMS pos *m/z* 324 $[M + H]^+$ (100); ESIMS neg *m/z* 322 $[M - H]^-$ (100); HRESIMS pos *m/z* 324.2482 $[M + H]^+$ (calcd for $C_{19}H_{34}NO_3$, 324.2538).

Melophlin O (13): yellow oil (MeOH); UV (MeOH) λ_{max} (log ϵ) 248 (3.79), 286 (3.99) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.68 (1H, q, J = 6.9 Hz, H-5), 2.96 (3H, s, NCH_3), 2.82 (2H, m, H_2-7), 1.64 (2H, m, H_2-8), 1.34 (3H, d, J = 6.9 Hz, 5-CH_3), 1.20−1.39 (15H, m, H_2-9-$H_{2[\omega-3]}$, $H_{\omega-2}$, $H_{2[\omega-1]}$), 0.85 (3H, t, J = 7.3 Hz, $H_{3[\omega]}$), 0.83 (3H, d, J = 6.3 Hz, [ω-2]-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 194.7 (s, C-4), 187.9 (s, C-6), 172.8 (s, C-2), 100.5 (s, C-3), 62.7 (d, C-5), 36.6 (t, $C_{\omega-3}$), 34.4 (d, $C_{\omega-2}$), 32.6 (t, C-7), 29.2−29.9 (4 × t, C-9-$C_{\omega-4}$), 29.7 (t, $C_{\omega-1}$), 27.1 (t, C-13), 26.2 (q, NCH_3), 26.0 (t, C-8), 19.2 (q, [ω-2]-CH_3), 14.8 (q, 5-CH_3), 11.4 (q, C_ω); EIMS *m/z* 323 $[M]^+$ (10), 182 (25), 169 (100), 154 (30); ESIMS pos *m/z* 324 $[M + H]^+$ (100); ESIMS neg *m/z* 322 $[M - H]^-$ (100); HRESIMS pos *m/z* 324.2482 $[M + H]^+$ (calcd for $C_{19}H_{34}NO_3$, 324.2538).

Hydrolysis and Derivatization Reactions. Compounds **1**, **8**, **10**, **11**, **12**, and **13** (1 mg each) were dissolved in 70 μL of acetone−H_2O (1:1) and treated with $NaIO_4$ (2.0 mg) and $KMnO_4$ (1.0 mg) at 5 °C for 30 min. The resulting mixture was treated with 2 N aqueous HCl (60 μL) at 100 °C for 2 h and neutralized with 1 M $NaHCO_3$ solution. Upon addition of *N*-(5-fluoro-2,4-dinitrophenyl-5)-L-leucinamide (FDLA, 0.4 mg in 380 μL of acetone), the reaction mixture was stirred at 37 °C for 2 h.[16] After quenching with 1 N aqueous HCl (80 μL), MeOH was added, and each sample was subjected to ESI LC/MS analysis.[17−19] The standard amino acids, *N*-methyl-L-alanine (300 μg) and *N*-methyl-DL-alanine (300 μg), were dissolved in acetone−H_2O (1:1) at a concentration of 10 μg/μL and derivatized with FDLA as described above.

ESI LC/MS. The FDLA derivatives of both enantiomers of *N*-methylalanine and the corresponding hydrolysates of compounds **1**, **8**, **10**, **11**, **12**, and **13** were analyzed by LC/MS according to the so-called "advanced Marfey's method".[17−19] The separation of the L- and D-FDLA derivatives of hydrolyzed compounds **1**, **8**, **10**, **11**, **12**, and **13** and of the standards L- and dl-*N*-methylalanine was performed on a Agilent 1100 series instrument using a 5 μm Eurospher-100 C18 column (2 mm i.d. × 150 mm). Acetonitrile (solvent A) and 10% MeCN in H_2O containing 0.1% formic acid (solvent B) were used as

mobile phases under a linear gradient elution mode at a flow rate of 0.2 mL/min using the following program (A:B, v/v): 0−5 min, 40:60; 5−35 min, linear gradient to 70:30; 35−45 min, 70:30. The mass spectrometer used was a Finnigan LCQ Deca (Finnigan-MAT, San Jose, CA). The retention times observed for (1-*N*-methyl-L-alanine-2,4-dinitrophenyl-5-)-L-leucinamide and (1-*N*-methyl-D-alanine-2,4-dinitrophenyl-5-)-L-leucinamide were 14.2 and 14.9 min, respectively. Two peaks with retention time of 14.2 and 14.9 min were observed upon LC/MS analysis for the derivatives of the hydrolysates of compounds **1**, **8**, **10**, **11**, **12**, and **13** with a ratio of approximately 2:1, 2:3, 2:1, 2:1, 2:1, and 2:1, respectively. The LC/MS peaks of both standard derivatives and all sample derivatives were observed at *m*/*z* 398.3 $[M + H]^+$ and 396.2 $[M - H]^-$ in ESI MS.

Chiral HPLC. Compounds **1** and **11** were subjected to HPLC on a Merck-Hitachi instrument (pump L-7100, detector L-7400) with CC 30/4 columns containing Nucleodex β-PM, Nucleodex γ-PM, and Nucleodex β-OH as chiral stationary phases, respectively. MeOH−H_2O containing 0.1% triethylammonium acetate, pH 4.0 (adjusted with acetic acid), 70:30 (v/v), was used as mobile phase at a flow rate of 0.7 mL/min according to the manufacturer's instructions. UV detection was at 283 nm. One sharp peak was observed on both β-PM and γ-PM columns, while one broad peak on the β-OH column was observed for both compounds **1** and **11**.

Agar Diffusion Assay. Susceptibility disks (5 mm in diameter) were impregnated with 5 and 10 μg of the isolated compounds dissolved in MeOH and placed on LB agar plates inoculated with the test bacteria *B. subtilis* 168, *S. aureus* ATCC 25923, and *E. coli* ATCC 25922. The plates were observed for zones of inhibition, after 24 h of incubation at 37 °C. The compounds were also assayed using *C. albicans* as test organism, which was inoculated on YPD agar plates, and zones of inhibition were recorded after 24 h of incubation at 27 °C. In all cases, for the controls containing only the respective amount of solvent, no zones of inhibition were observed.

Further Evaluation of Biological Activity. Antiproliferative activity was examined using cell lines HL-60, HELA, and TF-1 as described previously.[30] Activity against brine shrimp, *Artemia salina*, and insecticidal activity against larvae of *Spodoptera littoralis* were determined as described previously.[31,32]

Acknowledgment. Financial support by BMBF and by the Fonds der Chemischen Industrie (both to P.P.) is gratefully acknowledged. C.-Y.W. and B.-G.W. wish to thank the DAAD for scholarships. High-resolution mass spectra were from M. Nimtz (Gesellschaft für Biotechnologische Forschung, Braunschweig) and A. Berg (Hans-Knöll-Institut für Naturstofforschung, Jena). We are indebted to Y. Rusman, R. A. Edrada, and B. Steffan (all Institut für Pharmazeutische Biologie, Düsseldorf), and U. Hentschel (Institut für Molekulare Infektionsbiologie, Würzburg), for their help with the cytotoxicity and antibacterial assays.

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NP0202778