

Nuttingins A–F and Malonganenones D–H, Tetraprenylated Alkaloids from the Tanzanian Gorgonian *Euplexaura nuttingi*

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Six new tetraprenylated purine alkaloids, designated nuttingins A–F (**1–6**), were isolated together with three known malonganenones, A–C (**12–14**), and five new closely related malonganenones, D–H (**7–11**), from the gorgonian *Euplexaura nuttingi* collected in Pemba Island, Tanzania. The structures of the compounds were elucidated by interpretation of 1D and 2D NMR data including ¹⁵N chemical shifts obtained from ¹H–¹⁵N HMBC spectra. Nuttingins A–E (**1–5**) and malonganenones D–G (**7–10**) displayed inhibitory activity against both K562 and UT7 tumor cell lines, compounds **3–5** being the most active ones, approximately 3-fold more potent than the others. Compounds **1–5** and **7–11** also induce apoptosis in transformed mammalian cells at a concentration of 1.25 μg/mL.

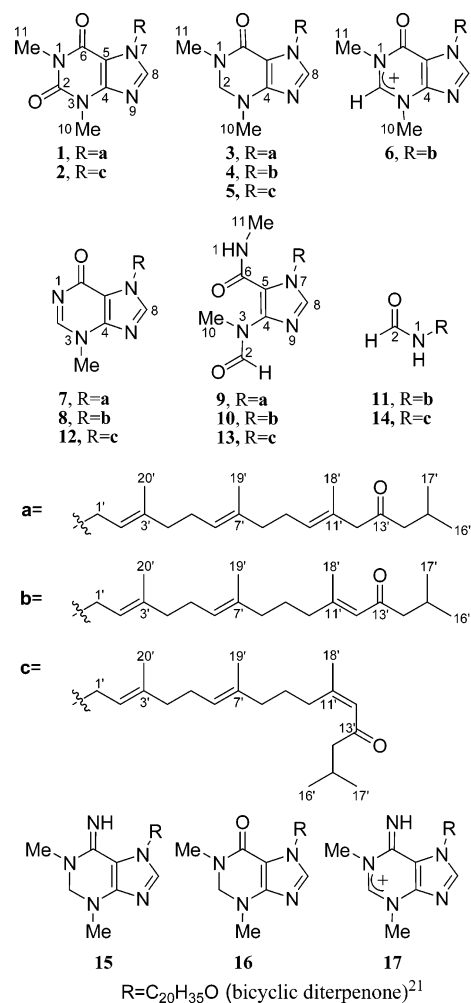
In continuation of our search for bioactive compounds from marine invertebrates^{1,2} we have examined a moderately active apoptosis-inducing extract of the gorgonian *Euplexaura nuttingi* (Kukenthal, 1919) collected in Uvinage, Pemba Island, Tanzania. Positive alkaloid coloring of the extract of this horny coral suggested N-atom-containing metabolites. Gorgonians, well known for production of isoprenoids and polyketide metabolites,^{3,4} are poor in N-atom-containing compounds, and only a few such compounds are known.^{5–10} A recent report on tetraprenylated purines, the malonganenones, which possess antiesophageal cancer activity, from the Mozambique gorgonian *Leptogorgia gilchristi*¹¹ encouraged us to report our findings from the Tanzanian gorgonian *E. nuttingi*.

Results and Discussion

The present report describes the isolation and structure elucidation of six new tetraprenylated purine alkaloids, designated nuttingins A–F (**1–6**), as well as eight malonganenones, tetraprenylated alkaloids, of which five (D–H) are new (compounds **7–11**) and three (A–C, compounds **12–14**) are known ones.¹¹ The compounds were isolated from the gorgonian *E. nuttingi* collected in Pemba Island, Tanzania.

The EtOAc–MeOH–H₂O (5:5:1) extract of the gorgonian was chromatographed over Sephadex LH-20 followed by silica gel VLC and RP-select B HPLC to yield compounds **1–14** (0.01%–0.08%, dry weight).

The FABMS spectrum of nuttingin A (**1**) exhibited a pseudo-molecular ion [M + H]⁺ at *m/z* 469.3168. A molecular formula of C₂₇H₄₀N₄O₃ was determined by HRMALDIMS, indicating 10 degrees of unsaturation. The NMR data (Tables 1 and 2) pointed clearly to a fused diterpene-*N,N*-dimethylpurine structure, *vide infra*. The diterpene portion embodies three trisubstituted double bonds (δ_C 143.2 C, 117.4 CH; 135.1 C, 123.5 CH; and 135.4 C, 129.3 CH) carrying three methyls (δ_C 16.9 CH₃, 16.4 CH₃, and 15.9 CH₃), as well as a ketone (δ_C 209.3 C) and an isopropyl termini (δ_C 22.5 CH₃ × 2). The structure of the remaining C₅H₇N₄O₂–*N,N*-dimethylxanthine moiety was established from its 1D (Tables 1 and 2) and 2D NMR data. This heterocyclic system was composed of a tetrasubstituted double bond (δ_C 148.8 C, 107.0 C), two carbonyl-amides (δ_C 155.3 C, 151.7 C), two *N*-methyls (δ_C 29.7 CH₃, 27.9 CH₃), and an imine CH group (δ_C 140.1 CH,



¹J_{CH} = 209 Hz, δ_H 7.52 s), all connected by HMBC correlations (Figure 1), and fully supported the suggested xanthine system as well as the N-7 location of the diterpene unit (correlation from H₂-1' to C-5).

¹⁵N NMR can be a powerful tool for structure determinations of N-containing natural products.^{12–14} The suitability of ¹⁵N NMR spectroscopy is attributed to the wide range of chemical shifts and its great sensitivity to structural and environmental changes. A major disadvantage of this spectroscopy is the extremely low sensitivity

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Table 1. ^{13}C NMR Spectroscopic Data for Nuttingins A–F (1–6)^a

position	1	2	3	4	5	6
2	151.7	151.7	70.6	70.6	70.6	151.5
4	148.8	148.8	157.8	157.8	157.8	146.8
5	107.0	107.0	107.0	107.0	107.0	114.1
6	155.3	155.3	160.9	160.9	160.9	151.7
8	140.1	140.1	138.8	138.8	138.8	145.6
10	27.9	27.9	35.4	35.4	35.4	37.7
11	29.7	29.7	32.2	32.2	32.2	36.0
1'	44.4	44.5	44.8	44.8	44.8	45.1
2'	117.4	117.3	118.9	118.5	118.9	118.7
3'	143.2	143.2	142.8	142.6	142.9	142.3
4'	39.4	39.8	40.0	39.8	39.9	39.6
5'	26.1	26.5	27.0	26.0	27.0	26.2
6'	123.5	123.5	124.6	124.5	124.5	124.5
7'	135.1	135.4	129.6	135.2	136.2	135.2
8'	39.2	40.2	40.1	40.7	40.2	40.0
9'	39.7	26.9	40.0	25.9	27.0	26.0
10'	129.3	33.9	130.2	33.4	34.2	39.0
11'	135.4	158.8	136.0	158.2	136.2	158.2
12'	54.3	124.1	55.2	124.2	124.9	124.0
13'	209.3	200.6	209.0	200.1	201.3	200.1
14'	50.5	53.4	51.2	53.2	54.2	53.1
15'	24.3	25.5	25.1	25.2	25.9	25.5
16', 17'	22.5	22.6	22.5	22.6	22.5	22.6
18'	16.4	25.9	16.8	19.0	25.9	19.1
19'	15.9	15.8	16.4	15.8	15.8	16.0
20'	16.5	16.5	16.9	16.5	16.4	16.7

^a Compounds 1–4 were measured at 400 MHz in CDCl_3 ; 5 and 6 in d_6 -DMSO.

of ^{15}N at the natural abundance level. However, the sensitivity issue can be overcome since inverse detection makes it possible to acquire one bond and long-range ^1H – ^{15}N correlations, circumventing the low sensitivity, and therefore obtaining ^{15}N chemical shifts. In previous papers we demonstrated the benefit of using ^{15}N NMR data (NH correlations $^2J_{\text{NH}}$ and $^3J_{\text{NH}}$ HMBC) for the structure determination of various N-atom-containing compounds^{1,2} including purine derivatives.^{14,15}

The ^1H – ^{15}N HMBC of **1** unequivocally confirmed the structure of the proposed heterocyclic ring system. $^2J_{\text{NH}}$ correlations observed between H-8 and two vicinal nitrogen atoms resonating at 168.7 and 229.7 ppm established these nitrogens to be N-7 (pyrrole sp^3) and N-9 (sp^2). Additional $^2J_{\text{NH}}$ correlations were observed between the two N-methyls to their neighbor N-atoms [i.e., between Me-10 and a nitrogen atom resonating at 113.6 (N-3) and between Me-11 and a second nitrogen atom resonating at 150.5 ppm (N-1) (Figure 2, Table 4)].

The structure elucidation of the tetraprenyl side chain of **1** was quite straightforward by interpretation of COSY and HMBC data (Figure 1). The COSY spectrum revealed the presence of four spin systems; *inter alia* a cross-peak was observed between the overlapping terminal methyl resonances (H₃-16' and H₃-17') and the methine proton (H-15'), which gave further COSY correlations with two methylene protons (H₂-14'). HMBC correlations from both H₂-14' and H-15' along with a correlation from the relatively low-field methylene H₂-12' (δ_{H} 3.00 s) to the carbonyl C-13' (δ_{C} 209.3 C) established the C-12' to C-17' segment. The latter segment was further extended by long-range 4J -COSY correlations between methylene H₂-12' and methyl CH₃-18' and between H-18' and H-10', as well as 3J -HMBC correlations from H₃-18' to both C-10' and C-12'. Contiguous methylene groups in the tetraprenyl side chain were assigned from the COSY correlations, and most informative was the HMBC experiment, which put together the side chain via two- and three-bond HMBC correlations of the three vinylic methyls (Figure 1). The appearance of the three vinyl methyl carbons upfield at 16.9, 16.4, and 15.9 ppm in the ^{13}C NMR spectrum established the stereochemistry of the three double bonds $\Delta^{2,6,10'}$ as *E* (in the case of *Z* geometry the vinyl methyl carbon should resonate around ~25 ppm because of the absence of a γ

effect; see below for compound **2**). A long-range 4J -COSY correlation observed between the methylene protons H₂-1' and H-8, $^3J_{\text{CH}}$ HMBC correlations between the latter protons and their corresponding carbons resonances (C-8 and C-1'), as well as ^1H – ^{15}N HMBC correlations between both H₂-1' and H-2' and N-7 (Figure 2), confirmed the tetraprenyl side-chain connection to N-7 of the xanthine moiety, determining the structure of the bicyclic ring system to be *N*(1),*N*(3)-dimethyl-*N*(7)-tetraprenylxanthine.

The mass spectrum of the second isolated compound, nuttingin B (**2**), suggested the same formula, $\text{C}_{27}\text{H}_{40}\text{N}_4\text{O}_3$, as that of **1**, implying, together with the NMR spectroscopic data, an isomeric structure. The major difference in the ^1H NMR data of **2** (Table 2) was the disappearance of the H₂-12' methylene (δ_{H} 3.00 s, 2H) and H-10' methine (δ_{H} 5.20 t) of **1** and the appearance of a singlet at 6.02 ppm and a broad triplet at 2.51 (2H) ppm, as well as in the ^{13}C NMR spectrum the shift of the resonances of one trisubstituted olefin to δ_{C} 158.8 C and 124.1 CH and the upfield shift of the carbonyl to δ_{C} 200.6 (Table 1), suggesting for **2** a trisubstituted α,β -enone functionality. Further confirmation of the latter moiety came from HMBC correlations between the olefinic protons H-12' (δ_{H} 6.02 s), H₂-14', and H-15' and carbonyl C-13'. Another difference in the NMR of **2** was the low-field resonance of one of the vinyl methyls (C-18', δ_{C} 25.9), suggesting a *Z* geometry for the $\Delta^{11'}$ double bond. The two other olefinic methyls C-19' and C-20' remained consistent with the *E* geometry for the $\Delta^{6'}$ and $\Delta^{2'}$ bonds, respectively. Nuttingins A and B represent a new addition to a very small but important group of naturally marine-occurring purine derivatives based on the xanthine nucleus that includes caffeine isolated from the Chinese gorgonian *Echinogorgia pseudosappo*¹⁶ and from the gorgonian *Echinomuraceae splendens*,¹⁷ theophylline isolated from the Bermudian sponge *Amphimedon viridis*,¹⁸ theobromine from the gorgonian *Echinomuraceae splendens*,¹⁷ and phidolopin isolated from the bryozoan *Phidolopora pacifica*.¹⁹

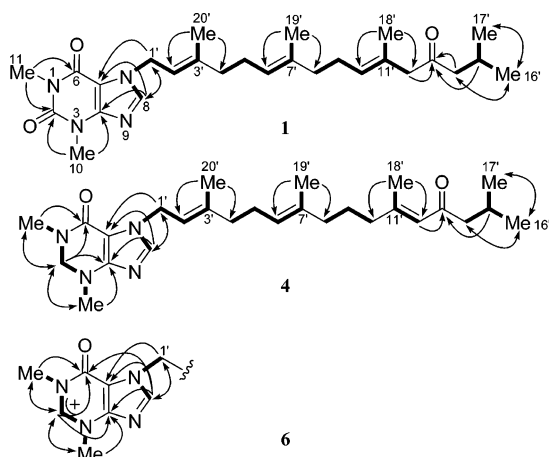
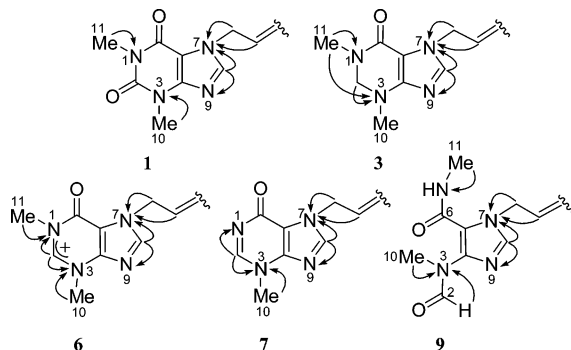
The HRMALDI mass spectrum of nuttingin C (**3**) exhibited a molecular ion $[\text{M} + \text{H}]^+$ at m/z 455.3384, suggesting, together with the ^{13}C NMR spectrum (Table 1), the molecular formula $\text{C}_{27}\text{H}_{42}\text{N}_4\text{O}_2$, indicating nine degrees of unsaturation. Inspection of the ^1H and ^{13}C NMR spectra (Tables 1 and 2) revealed a fused purine–diterpene structure, the diterpene portion being identical to the tetraprenyl side chain of nuttingin A (**1**). Nuttingins A (**1**) and C (**3**), however, differ in the heterobicyclic moiety. In the ^1H NMR of **3** the two N-methyl singlets as well as H-8 shifted upfield, in comparison to **1**, to δ_{H} 2.90, 2.97, and 7.21, respectively. Noticeable also was the appearance of a new methylene at δ_{H} 4.27 (2H) that gave rise to a triplet at δ_{C} 70.6, in the ^{13}C NMR spectrum, occurring instead of one of the two purine carbonyls of **1**. HMBC correlations between both N-methyls and C-2 (δ_{C} 70.6 CH₂, δ_{H} 4.27 s), long-range 4J -COSY correlations between each N-methyl and H₂-2, and the low field of the latter methylene established its position between two nitrogen atoms. Further $^3J_{\text{CH}}$ -HMBC correlations from Me-10 and H₂-2 to C-4 and from Me-11 and H₂-2 to the purine carbonyl (C-6), as well as from H-8 to C-4 and C-5, established the bicyclic moiety of **3** as shown in Figure 1. Nuttingin C (**3**) possesses a reduced purine skeleton unprecedented in marine sources. The most closely related bicyclic moiety is the one in the marine-adenine derivative agelasimine-B (**15**).²⁰ Recognizable as having the same ring system is the synthetic intermediate of agelasimine-B (**16**).²¹

Two $^2J_{\text{NH}}$ correlations were observed in the ^1H – ^{15}N HMBC experiment of **3**, between H-8 and its two vicinal nitrogen atoms resonating at 169.5 and 226.3 ppm (Table 4). In addition $^2J_{\text{NH}}$ and $^3J_{\text{NH}}$ correlations were seen from both H₂-1' and H-2' to the nitrogen atom resonating at 169.5 ppm, together confirming the position of the latter nitrogen atoms as N-7 and N-9, respectively. A $^2J_{\text{NH}}$ correlation from one N-methyl group (11) to the N-atom resonating at 93.2 ppm established this N-atom as the conjugated amide N-atom, N-1. Assignment of the fourth N-atom was achieved on

Table 2. ^1H NMR Spectroscopic Data for Nuttingins A–F (1–6)^a

position	1	2	3	4	5	6
2			4.27, s	4.28, s	4.29, s	9.93, s
8	7.52, s	7.53, s	7.21, s	7.22, s	7.23, s	8.62, s
10	3.40, s	3.41, s	2.90, s	2.90, s	2.91, s	3.97, s
11	3.58, s	3.58, s	2.97, s	2.98, s	2.98, s	3.65, s
1'	4.92, d (7.2)	4.92, d (7.2)	4.82, d (7.1)	4.82, d (7.1)	4.84, d (7.0)	5.04, d (6.8)
2'	5.43, t (7.2)	5.43, t (7.2)	5.40, dt (6.7, 0.9)	5.40, t (6.5)	5.42, t (6.7)	5.42, t (7.0)
4'	2.05, m	2.01, m	2.03, m	2.02, m	2.03, m	2.03, m
5'	2.12, m	2.11, m	2.12, m	2.09, m	2.17, m	2.15, m
6'	5.06 t, (6.1)	5.08, t (6.1)	5.08, t (6.3)	5.06, t (6.3)	5.08, t (6.7)	5.05, m
8'	2.07, m	2.01, t (5.9)	2.04, m	2.07, m	2.04, m	2.05, m
9'	2.12, m	1.51, m	2.11, m	1.52, m	1.51, m	1.47, m
10'	5.20, t (6.0)	2.51, br t (7.9)	5.20, dt (6.3, 0.9)	2.50, br t (7.9)	2.09, m	2.12, m
12'	3.00, s	6.02, s	2.98, s	6.01, s	6.03, s	6.08, s
14'	2.27, d (6.9)	2.26, d (7.0)	2.26, d (6.9)	2.24, d (7.0)	2.28, d (6.9)	2.26, d (6.9)
15'	2.18, m	2.12, m	2.10, m	2.13, m	2.07, m	2.07, m
16', 17'	0.90, d (6.6)	0.89, d (6.7)	2.87, d (6.7)	0.90, d (6.6)	0.92, d (6.5)	0.85, d (6.6)
18'	1.60, s	1.86, s	1.59, s	1.84, s	2.11, s	2.02, s
19'	1.59, s	1.60, s	1.58, s	1.58, s	1.58, s	1.54, s
20'	1.78, s	1.78, s	1.74, s	1.73, s	1.76, s	1.79, s

^a Compounds 1–4 were measured at 400 MHz in CDCl_3 ; 5 and 6 in d_6 -DMSO.

**Figure 1.** Key COSY and HMBC correlations for 1, 4, and 6.**Figure 2.** ^1H – ^{15}N HMBC correlations measured for the different heterocyclic moieties.

the basis of the distinction between the two *N*-methyl groups by the HMBC experiment (Figure 1, as shown for 4). $^2J_{\text{NH}}$ and $^4J_{\text{NH}}$ correlations from both H-2 and Me-11 to a nitrogen atom resonating at 53.8 ppm established this atom as the tertiary N-3 (Figure 2, Table 4).¹²

Compounds 4 and 5, like 3, were both assigned by HRM-ALDIMS the molecular composition $\text{C}_{27}\text{H}_{42}\text{N}_4\text{O}_2$ ($[\text{M} + \text{H}]^+$, m/z 455.3383), implying, together with the NMR spectroscopic data, isomeric structures. Both 4 and 5 possess, according to the NMR data, the same bicyclic moiety as 3 (Tables 1 and 2). Differences were only seen in the tetraprenyl side chain. Comparing the δ_{C} values of the side chain of 4 and 2 (Table 1) revealed that the only difference was the vinylic methyl C-18' that was relatively high

field in 4 (19.0 ppm), suggesting *E* geometry for the $\Delta^{11'}$ bond. The somewhat low-field proton signal of the latter methyl (δ_{H} 1.84 s), relative to the other vinylic methyls in the side chain, is attributed to a downfield shift by the neighboring carbonyl group. In compound 5, however, the tetraprenyl side chain was found to be identical to that of compound 2.

Compounds 3–5 are unstable, as was concluded by monitoring their NMR spectra in CDCl_3 (Tables 1 and 2) or d_6 -DMSO. Within 3–4 days in the NMR tube, the purine system of nuttingins C–E (3–5) changes into the cationic system found in compound 6 (Scheme 1). Characteristic for this oxidation were the changes of the two *N*-methyl signals (Tables 1 and 2). In the case of 4 the mixture was separated after 4 days by HPLC to afford compounds 4 and 6. Compound 6, nuttingin F, was also obtained from the crude gorgonian extract. It is unknown at what stage, in the organism or during isolation, the oxidation of 4 to 6 takes place. A similar but rapid oxidation of a reduced purine to the cationic system was reported synthetically with DDO as the oxidant.²¹

The FAB mass spectrum of the charged molecule (6) exhibited a molecular ion $[\text{M} + \text{H}]^+$ at m/z 454.3308. The molecular formula $\text{C}_{27}\text{H}_{41}\text{N}_4\text{O}_2$ was determined by HRM-ALDIMS. The ^1H NMR revealed two unusually low-field *N*-methyls at δ_{H} 3.97 and 3.65 as well as two single low-field protons at δ_{H} 9.93 and 8.62. Noticeable also was the disappearance of the H-2 methylene singlet of 3–5. Major changes in the ^{13}C NMR of 6 were the chemical shifts of two heterocyclic sp^2 carbons, the latter bearing low-field protons (HSQC), at δ_{C} 151.1 and 145.6. HMBC and 4J -COSY correlations between the two *N*-methyls and the low-field proton (δ_{H} 9.93) (Figure 1) determined the latter's C-2 position. Further support for the location of the C(2)-immonium cation came from HMBC correlations from both H-2 and Me-11 to the purine carbonyl C-6 (δ_{C} 151.7) and from H-2 and Me-10 to the singlet carbon at δ_{C} 146.8 (C-4). The low-field proton resonances of both *N*-methyls and H-2 suggested that the positive charge of the immonium ion is spread over the two nitrogen atoms. The above data, together with additional HMBC correlations from H-8 to C-4, C-5 (3J) and to C-6 (4J), established the bicyclic moiety as described in Figure 1. Further support for the structure came from ^1H – ^{15}N HMBC correlations between H-8 and two nitrogen atoms resonating at 172.5 (N-7) and 233.2 ppm (N-9) and from both H-2' and H-2'' to N-7 (Figure 2). $^2J_{\text{NH}}$ correlations were also observed for both *N*-methyls, i.e., between Me-10 and a nitrogen atom resonating at 143.6 ppm (N-3), from Me-11 to a second nitrogen atom resonating at 170.0 ppm (N-1), and from H-2 to both latter vicinal nitrogen atoms (Figure 2, Table 4). The relatively low field of N-1 and N-3 compared to the corresponding δ_{N} values of 1 is expected from

Table 3. ^1H and ^{13}C NMR Spectroscopic Data for Malonganenones D–H (7–11)^a

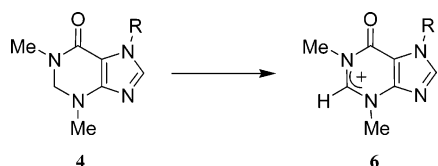
position	7		8		9		10		11	
	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}
1						7.98, br q (4.4)		8.08, br q (4.4)		
2	147.2	8.02, s	147.5	8.19, s	163.5	8.15, s	163.4	8.13, s	161.6	8.16, s
4	147.3		147.4		141.5		141.2			
5	114.0		115.0		118.3		117.8			
6	162.8		162.1		161.1		161.2			
8	139.9	7.67, s	140.3	7.70, s	137.6	7.63, s	137.3	7.63, s		
10	34.6	3.82, s	35.0	3.86, s	32.5	3.04, s	32.6	3.05, s		
11					27.2	2.67, d (4.6)	27.0	2.68, d (4.8)		
1'	44.3	5.09, d (7.3)	44.4	5.09, d (7.1)	45.0	4.70, d (6.9)	45.1	4.69, d (7.2)	36.5	3.90, dd (12.5, 6.2)
2'	117.7	5.47, t (7.0)	117.5	5.47, t (7.0)	120.5	5.22, t (7.0)	120.3	5.21, t (7.0)	120.2	5.20, t (6.5)
3'	143.2		143.6		141.3		141.3		141.2	
4'	39.3	2.10, m	39.4	2.15, m	39.9	1.99, m	39.6	2.02, m	41.1	2.04, m
5'	26.1	2.10, m	26.0	2.18, m	26.9	2.05, m	26.7	2.04, m	26.6	2.08, m
6'	123.5	5.06, m	123.5	5.08, m	124.9	5.04, t (6.1)	124.8	5.03, t (6.1)	124.4	5.09, t (6.3)
7'	135.4		135.2		135.7		135.5		135.9	
8'	39.2	2.00, m	40.7	2.08, m	39.9	1.99, m	40.5	2.00, m	39.5	2.05, m
9'	26.6	2.00, m	25.7	2.13, m	27.0	2.05, m	26.2	1.47, m	26.2	1.53, m
10'	129.2	5.20, t (6.5)	33.4	2.49, t (8.2)	129.8	5.15, t (6.6)	37.3	1.87, t (7.4)	33.9	2.50, t (8.0)
11'	128.9		158.2		130.3		158.6		160.0	
12'	54.3	2.99, s	123.9	6.01, s	54.6	2.98, s	124.3	6.06, s	125.0	6.03, s
13'	209.3		201.2		209.7		202.0		201.0	
14'	50.5	2.27, d (6.9)	53.4	2.27, d (6.9)	51.0	2.25, d (6.9)	53.5	2.27, d (7.2)	54.2	2.28, d (7.0)
15'	24.4	2.08, m	25.1	2.11, m	24.9	2.05, m	25.5	1.98, m	25.6	2.17, m
16', 17'	22.5	0.88, d (6.5)	22.6	0.90, d (6.5)	23.5	0.80, d (6.6)	23.2	0.82, d (6.8)	23.4	0.92, d (6.5)
18'	16.5	1.58, s	19.2	2.05, s	17.4	1.50, s	19.5	2.00, s	20.0	2.11, s
19'	15.9	1.57, s	15.8	1.79, s	16.9	1.52, s	16.4	1.51, s	16.6	1.59, s
20'	16.3	1.78, s	16.5	1.58, s	17.3	1.57, s	16.8	1.66, s	17.0	1.68, s

^a Compounds **7**, **8**, and **11** were measured at 400 MHz in CDCl_3 ; **9** and **10** in d_6 -DMSO.

Table 4. ^{15}N NMR Data for Nuttingins **1**, **3**, and **6** and Malonganenones **7** and **9**^a

position	1	3	6	7	9
1	150.5	93.2	170.0	260.9	103.9
3	113.6	53.8	143.6	137.1	117.7
7	168.7	169.5	172.5	169.0	173.7
9	229.7	226.3	232.2	232.3	248.8

^a ^{15}N chemical shifts deduced from ^1H – ^{15}N HMBC experiments.

Scheme 1. Air Oxidation of Nuttingin D (**4**) to Nuttingin F (**6**)

the positive charge spread over the two nitrogens. The similar downfield shift of both N-1 and N-3 suggests a similar partial positive charge on both (Table 4).¹⁴ To the best of our knowledge, a C-2 positively charged purine is naturally unprecedented. A synthetic closely related positively charged purine derivative was reported as an intermediate in the total synthesis of agelasimine-B (**17**).²¹

Together with the nuttingins (**1**–**6**) were also isolated three known malonganenones (A–C)¹² and five new ones (D–H). The FAB mass spectrum of malonganenones D and E (**7**, **8**) exhibited a molecular ion $[\text{M} + \text{Na}]^+$ at m/z 461.2890. The molecular formula was established as $\text{C}_{26}\text{H}_{38}\text{N}_4\text{O}_2$ from HRMALDIMS and ^{13}C NMR data, suggesting 10 degrees of unsaturation. The ^{13}C and ^1H NMR spectra of **7** and **8** (Table 3) were very similar to those of malonganenone A (**12**), recently reported from the Mozambique (several hundred km to the south) gorgonian *Leptogorgia gilchristi*.¹¹ An identical molecular formula, together with the NMR spectroscopic data, implied isomeric structures. The spectra of **7** revealed the absence of the H-12' singlet at 6.02 ppm, compared to malonganenone A (**12**), two additional methylene groups [δ_{H} 5.20 t (2H), 2.99 s (2H)], and also a downfield shift of the carbonyl ketone to δ_{C} 209.3. The 1D (Tables 1 and 2) and 2D NMR

data of **7** confirmed the assumption that compound **7** is composed of the 3,7-disubstituted hypoxanthine bicyclic ring system of malonganenone A (**12**) and a tetraprenyl side chain as in compound **1**, with the same geometries of the three olefins. The difference in compound **8** was the relatively low-field vinylic methyl C-18' (19.2 ppm), compared to **1** and **8**, suggesting the *E* geometry for $\Delta^{11'}$, as in compound **4**. Confirmation of the bicyclic ring system of **8**, i.e., a 3,7-disubstituted hypoxanthine, came from ^1H – ^{15}N HMBC correlations between H-8 and two nitrogen atoms resonating at 169.0 ppm (N-7) and 232.3 ppm (N-9) (sp^3 and sp^2 N-atoms, respectively) and from both H-2-1' and H-2' to N-7. $^2J_{\text{NH}}$ correlations were also observed between H-2 and two nitrogen atoms resonating at 137.1 (N-3, sp^3) and 260.9 ppm (N-1, sp^2) and between the N-methyl and N-3 (Figure 2).

Malonganenones F and G (**9**, **10**) were both assigned the molecular composition $\text{C}_{27}\text{H}_{43}\text{N}_4\text{O}_3$ (HRMALDIMS, m/z 471.3340), indicating nine degrees of unsaturation. The molecular formula together with the NMR spectroscopic data (Table 3) suggested a structure isomeric to malonganenone B (**13**).¹¹ The latter data suggested that **9** and **10** are isomeric and differ from malonganenone B (**13**) similar to the way that compounds **7** and **8** differ from malonganenone A (**12**), namely, by the structure of the tetraprenyl side chain. Both **9** and **10** possess the same trisubstituted imidazole ring carrying, in addition to the diterpene portion, an *N*-methylamide and an *N*-methylformamide substituent. The ^1H – ^{15}N HMBC experiment of **9** confirmed the trisubstituted imidazole structure by two $^2J_{\text{NH}}$ correlations between H-8 and two vicinal nitrogen atoms resonating at 173.7 and 248.8 ppm, characteristic for the imidazole nitrogen atoms N-7 and N-9, respectively.¹² Additionally, the *N*-methylformamide substituent was confirmed by correlations from both H-2 and Me-10 to an amide nitrogen atom resonating at 117.7 (N-3). $^2J_{\text{NH}}$ correlation observed from Me-11 to its neighbor amide N-atom (N-1), resonating at 103.9, and the residual $^1J_{\text{NH}}$ coupling of NH-1 (90 Hz) determined the *N*-methylamide functionality (Figure 2, Table 4).

The molecular formula of the fifth new malonganenone (H, **11**) was readily deduced from HRCIMS as $\text{C}_{21}\text{H}_{36}\text{NO}_2$ ($[\text{M} + \text{H}]^+$ m/z 334.2750), indicating five degrees of unsaturation. Careful analysis

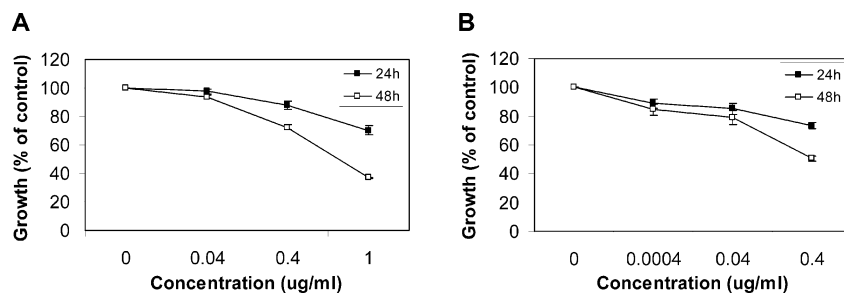


Figure 3. Nuttingins C–E (3–5) inhibit growth of K562 (A) and UT7 (B) cell lines. Cells were exposed to the indicated amounts of 3–5 for 24 and 48 h. Cell growth is expressed as a percentage of the control. Each value represents the mean \pm SD of 3 experiments.

of the COSY and HMBC spectra of **11** confirmed the presence of the same tetraprenyl group as found in **4**, **6**, **8**, and **10**, leaving a CH_2NO unit, rather than an imidazole or purine unit, to be assigned. The HSQC spectrum revealed a one-bond coupling between the carbonyl group at δ_{C} 161.6 CH and the deshielded proton at δ_{H} 8.16 s, suggesting a terminal formamide group, thus making compound **11** a structure isomeric to malonganenone C (**14**) differing in the geometry of the Δ^{11} double bond.

The structures of compounds **12**–**14** were found to be identical to malonganenones A–C, tetraprenylated alkaloids recently reported from the gorgonian *Leptogorgia gilchristi*.¹¹

Nuttingins A–E (**1**–**5**) and malonganenones D–G (**7**–**10**) have been found to inhibit growth of K562²² and UT7²³ cells. The tests were done on mixtures of compounds **1**–**2**, **3**–**5**, **7**–**8**, and **9**–**10**, as there was no real difference between the activities of compounds differing only in the side chain, e.g., compounds **3**, **4**, and **5**. To investigate the potential effects of the compounds on cell proliferation, two different human leukemia cell lines, K562 and UT7, were used as targets and treated with various concentrations of compound **1**–**5** and **7**–**10** for 24 and 48 h. As shown in Figure 3, compounds **3**–**5** induced inhibition of cell growth in K562 (A) and UT7 (B), in a dose- and time-dependent manner. UT7 cells displayed a greater sensitivity to compounds **3**–**5**, as compared to K562. Namely, at 0.4 $\mu\text{g}/\text{mL}$ compounds **3**–**5** induced 50% inhibition of cell growth in UT7 cells (B) and 30% in K562 cells (A), after 48 h of exposure to the compounds. Compounds **1**–**2**, **7**–**8**, and **9**–**10** also displayed inhibitory activity on proliferation of both cell lines, although they were approximately 3-fold less potent than compounds **3**–**5** (data not shown). Compounds **1**–**5** and **7**–**11** induce apoptosis in transformed mammalian cells at a concentration of 1.25 $\mu\text{g}/\text{mL}$.²⁴

Experimental Section

General Experimental Procedures. Optical rotations were obtained with a Jasco P-1010 polarimeter. IR spectra were obtained with a Bruker FTIR Vector 22 spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance-400 spectrometer. ^1H , ^{13}C , COSY, HSQC, and HMBC were recorded using standard Bruker pulse sequences. Chemical shifts are reported in ppm and are referenced to residual solvent resonances (CDCl_3 δ_{H} 7.26, δ_{C} 77.0 or d_6 -DMSO δ_{H} 2.50, δ_{C} 40.0). The ^1H – ^{15}N HMBC experiments were optimized for a delay of 65 ms, and the ^{15}N chemical shifts are reported with respect to liquid NH_3 as the reference standard. FABMS and CIMS measurements were recorded on a Fisons, Autospec Q instrument. HRMALDIMS measurements were recorded on an Applied Biosystem Voyager DE-STR MALDI TOF instrument.

Biological Material. The octocoral *Euplexaura nuttingi* (Kukenthal, 1919) was collected in Uvinae, Pemba Island, Tanzania, 5°08'06" S, 39°38'58" E (December 2, 2004). A voucher specimen is deposited at the Zoological Museum, Tel Aviv University, Israel (ZMTAU CO 32699). This soft coral was collected from a reef slope at a depth of 18–25 m, inhabited also by a large variety of other octocorals, sponges, and tunicates. The brown-red colonies of *E. nuttingi* commonly grow on vertical substrata exposed to strong currents. Prior to the present collection this species was recorded in the Moluccas and the Red Sea. Both *E. nuttingi* and *L. gilchristi* are similar in appearance.

Extraction and Isolation. Freeze-dried *E. nuttingi* (10 g) was homogenized and extracted with EtOAc–MeOH– H_2O (5:5:1). The organic extract was concentrated to yield a crude extract (1.1 g). The crude extract was chromatographed on a Sephadex LH-20 column, eluting with *n*-hexane–MeOH– CHCl_3 (2:1:1) and then subjected to VLC over Si gel, using *n*-hexane with increasing proportions of EtOAc as eluent followed with increasing proportions of MeOH. The different fractions from the Si gel column were subjected to RP-HPLC reversed-phase separation (Merck LiChrospher 60 RP-select B Hibar repacked column, 5 μm , 250 \times 25 mm) using a mixture of 70% CH_3CN –30% H_2O + 0.01% TFA as the eluent. The fraction eluted by VLC with 50% EtOAc afforded in the HPLC two compounds: **11** (2.0 mg, 0.02% dry weight) and **14** (1.0 mg, 0.01% dry weight). The fraction eluted with 60% EtOAc was separated to give two compounds: **1** (2.0 mg, 0.02% dry weight) and **2** (2.0 mg, 0.02% dry weight). The fraction eluted with EtOAc gave the following three compounds: **3** (6.7 mg, 0.07% dry weight), **4** (8.3 mg, 0.08% dry weight), and **5** (2.0 mg, 0.02% dry weight). The fraction eluted with 2% MeOH in EtOAc was separated to give three compounds: **9** (5.3 mg, 0.05% dry weight), **10** (3.0 mg, 0.03% dry weight), and **13** (3.7 mg, 0.04% dry weight). The fraction eluted with 50% MeOH was separated to give three compounds: **7** (6.0 mg, 0.06% dry weight), **8** (1.0 mg, 0.01% dry weight), and **12** (6.0 mg, dry weight). Compound **6** was isolated from the mixture that developed in the NMR tube of compound **4**. The mixture was subjected to HPLC (same conditions as mentioned above), affording 1.3 mg of pure compound **6**. The optical rotation of all compounds was zero.

Nuttingin A (1): colorless oil; IR (CH_2Cl_2) ν_{max} 2960, 1704, 1658, 1548, 1451, 1382, 1218, 1213 cm^{-1} ; ^1H and ^{13}C NMR, see Tables 1 and 2; HRMALDIMS $[\text{M} + \text{H}]^+ m/z$ 469.3168 (calcd for $\text{C}_{27}\text{H}_{41}\text{N}_4\text{O}_3$, 469.3173).

Nuttingin B (2): colorless oil; IR (CH_2Cl_2) ν_{max} 2959, 1705, 1644, 1548, 1462, 1384, 1218, 1212 cm^{-1} ; ^1H and ^{13}C NMR, see Tables 1 and 2; HRMALDIMS $[\text{M} + \text{H}]^+ m/z$ 469.3170 (calcd for $\text{C}_{27}\text{H}_{41}\text{N}_4\text{O}_3$, 469.3173).

Nuttingin C (3): colorless oil; IR (CH_2Cl_2) ν_{max} 2960, 2930, 1659, 1450, 1383, 1214 cm^{-1} ; ^1H and ^{13}C NMR, see Tables 1 and 2; HRMALDIMS $[\text{M} + \text{H}]^+ m/z$ 455.3384 (calcd for $\text{C}_{27}\text{H}_{42}\text{N}_4\text{O}_2$, 455.3386).

Nuttingin D (4): colorless oil; IR (CH_2Cl_2) ν_{max} 2960, 1642, 1451, 1383, 1217, 1212 cm^{-1} ; ^1H and ^{13}C NMR, see Tables 1 and 2; HRMALDIMS $[\text{M} + \text{H}]^+ m/z$ 455.3383 (calcd for $\text{C}_{27}\text{H}_{42}\text{N}_4\text{O}_2$, 455.3386).

Nuttingin E (5): colorless oil; IR (CH_2Cl_2) ν_{max} 2960, 1644, 1450, 1383, 1218, 1214 cm^{-1} ; ^1H and ^{13}C NMR, see Tables 1 and 2; HRMALDIMS $[\text{M} + \text{H}]^+ m/z$ 455.3383 (calcd for $\text{C}_{27}\text{H}_{41}\text{N}_4\text{O}_3$, 455.3386).

Nuttingin F (6): colorless oil; IR (CH_2Cl_2) ν_{max} 2958, 2360, 1728, 1677, 1214, 1138 cm^{-1} ; ^1H and ^{13}C NMR, see Tables 1 and 2; HRMALDIMS $[\text{M} + \text{H}]^+ m/z$ 454.3308 (calcd for $\text{C}_{27}\text{H}_{41}\text{N}_4\text{O}_2$, 454.3310).

Malonganenon D (7): colorless oil; IR (CH_2Cl_2) ν_{max} 2960, 1644, 1452, 1384, 1218, 1212, 1030 cm^{-1} ; ^1H and ^{13}C NMR, see Table 3; HRMALDIMS $[\text{M} + \text{Na}]^+ m/z$ 461.2890 (calcd for $\text{C}_{26}\text{H}_{38}\text{N}_4\text{O}_2\text{Na}$, 461.2887).

Malonganenon E (8): colorless oil; IR (CH_2Cl_2) ν_{max} 2959, 1644, 1462, 1384, 1218, 1212, 1096 cm^{-1} ; ^1H and ^{13}C NMR, see Table 3; HRMALDIMS $[\text{M} + \text{Na}]^+ m/z$ 461.2890 (calcd for $\text{C}_{26}\text{H}_{38}\text{N}_4\text{O}_2\text{Na}$, 469.2887).

Malonganenon F (9): colorless oil; IR (CH₂Cl₂) ν_{\max} 2960, 1665, 1564, 1454, 1213 cm⁻¹; ¹H and ¹³C NMR, see Table 3; HRMALDIMS [M + H]⁺ m/z 471.3340 (calcd for C₂₇H₄₃N₄O₃, 471.3335).

Malonganenon G (10): colorless oil; IR (CH₂Cl₂) ν_{\max} 2960, 1665, 1612, 1451, 1358, 1218, 1212 cm⁻¹; ¹H and ¹³C NMR, see Table 3; HRMALDIMS [M + H]⁺ m/z 471.3340 (calcd for C₂₇H₄₃N₄O₃, 471.3335).

Malonganenon H (11): colorless oil; IR (CH₂Cl₂) ν_{\max} 3441, 2931, 1682, 1611, 1450, 1384, 1212, 1141 cm⁻¹; ¹H and ¹³C NMR, see Table 3; HRCIMS [M + H]⁺ m/z 334.2750 (calcd for C₂₁H₃₆NO₂, 334.2746).

Cell Culture. The human leukemia cell lines K562²² and UT7²³ were grown in RPMI 1640 and IMDM medium, respectively, supplemented with 10% fetal bovine serum (FBS), 2 mM l-glutamine, 100 μ g/mL streptomycin, and 100 μ g/mL penicillin. UT7 cells were cultured in the presence of 2 units/mL erythropoietin (Eprex). Cells were maintained at 37 °C in a 5% CO₂ humidified incubator.

Colorimetric MTT Assay. Cells (4 × 10³) were seeded in triplicate into 96-well, flat-bottom culture plates and grown in the presence of the compounds at different concentrations for 24 and 48 h. Untreated cells served as a control. After incubation with the compound, cell growth was determined using the colorimetric methylthiazole tetrazolium bromide (MTT) assay.²⁵ Briefly, MTT was added to a final concentration of 5 μ g/mL to each well and further incubated for 4 h at 37 °C. After complete solubilization of the dye by acid/alcohol (0.04 N HCl in 2-propanol), plates were read at 570 nm in an ELISA reader, reference 690 nm. Growth of cells exposed to treatment was calculated as the percent of OD of the compound-treated cells to that of the nontreated cells.

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Supporting Information Available: ¹H and ¹³C NMR spectra for compounds **2**, **4**, and **6**, including HMBC and ¹⁵N-HMBC for compound **2**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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