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 ^{15}N chemical shifts obtained from $^{1}H-^{15}N$ 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.

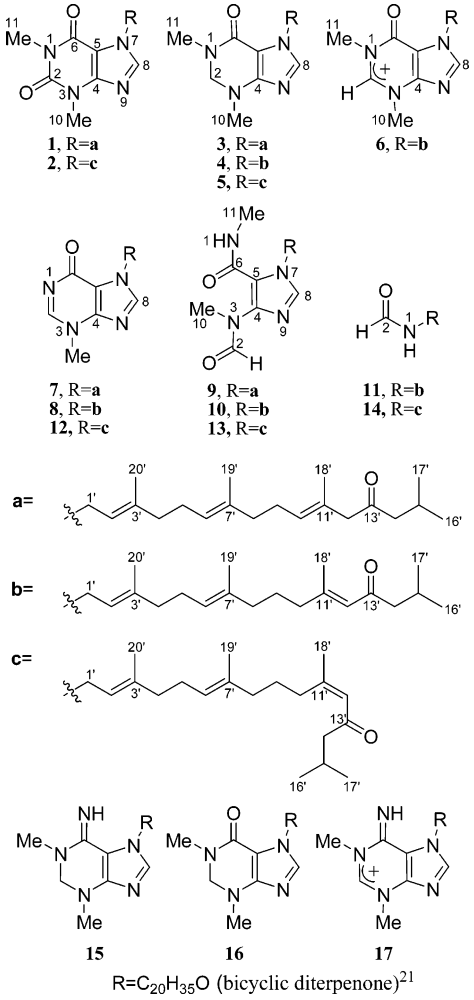
In continuation of our search for bioactive compounds from marine invertebrates[1,2] we have examined a moderately active apoptosis-inducing extract of the gorgonian *Euplexaura nuttingi* (Kukenthal, 1919) collected in Uvinage, Pemba Island, Tanzania. Positive alkaloid coloring of the extract of this 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−H_2O (5:5:1) extract of the gorgonian was chromotographed 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 pseudomolecular ion $[M + H]^+$ at *m/z* 469.3168. A molecular formula of $C_{27}H_{40}N_4O_3$ 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 CH_3, 16.4 CH_3, and 15.9 CH_3), as well as a ketone (δ_C 209.3 C) and an isopropyl termini (δ_C 22.5 CH_3 × 2). The structure of the remaining $C_5H_7N_4O_2$−*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 CH_3, 27.9 CH_3), and an imine CH group (δ_C 140.1 CH, $^{1}J_{C,H}$ = 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 H_2-1′ to C-5).



^{15}N NMR can be a powerful tool for structure determinations of N-containing natural products.[12−14] The suitability of ^{15}N 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 ^{1}H−^{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 $^2J_{NH}$ and $^3J_{NH}$ HMBC) for the structure determination of various N-atom-containing compounds[1,2] including purine derivatives.[14,15]

The ^{1}H−^{15}N HMBC of **1** unequivocally confirmed the structure of the proposed heterocyclic ring system. $^2J_{NH}$ 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 $^2J_{NH}$ 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 (H_3-16′ and H_3-17′) and the methine proton (H-15′), which gave further COSY correlations with two methylene protons (H_2-14′). HMBC correlations from both H_2-14′ and H-15′ along with a correlation from the relatively low-field methylene H_2-12′ (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 H_2-12′ and methyl CH_3-18′ and between H-18′ and H-10′, as well as ^3J-HMBC correlations from H_3-18′ 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 H_2-1′ and H-8, $^3J_{CH}$ HMBC correlations between the latter protons and their corresponding carbons resonances (C-8 and C-1′), as well as ^{1}H−^{15}N HMBC correlations between both H_2-1′ 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, $C_{27}H_{40}N_4O_3$, as that of **1**, implying, together with the NMR spectroscopic data, an isomeric structure. The major difference in the ^{1}H NMR data of **2** (Table 2) was the disappearance of the H_2-12′ 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), H_2-14′, 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 pseudossapo*[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 $[M + H]^+$ at *m*/*z* 455.3384, suggesting, together with the ^{13}C NMR spectrum (Table 1), the molecular formula $C_{27}H_{42}N_4O_2$, indicating nine degrees of unsaturation. Inspection of the ^{1}H 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 ^{1}H 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 CH_2, δ_H 4.27 s), long-range ^4J-COSY correlations between each *N*-methyl and H_2-2, and the low field of the latter methylene established its position between two nitrogen atoms. Further $^3J_{CH}$-HMBC correlations from Me-10 and H_2-2 to C-4 and from Me-11 and H_2-2 to the purine carbonyl (C-6), as well as from H-8 to C-4 and C-5, established the bicylic 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 $^2J_{NH}$ correlations were observed in the ^{1}H−^{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 $^2J_{NH}$ and $^3J_{NH}$ correlations were seen from both H_2-1′ 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 $^2J_{NH}$ 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. ^{1}H 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)	0.87, 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,	1.54, s
20′	1.78, s	1.78, s	1.74, s	1.73, s	1.76,	1.79, s

[a] Compounds **1**−**4** were measured at 400 MHz in $CDCl_3$; **5** and **6** in d_6-DMSO.

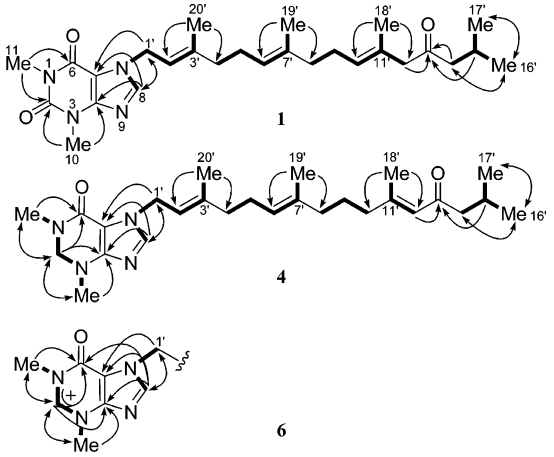


Figure 1. Key COSY and HMBC correlations for **1**, **4**, and **6**.

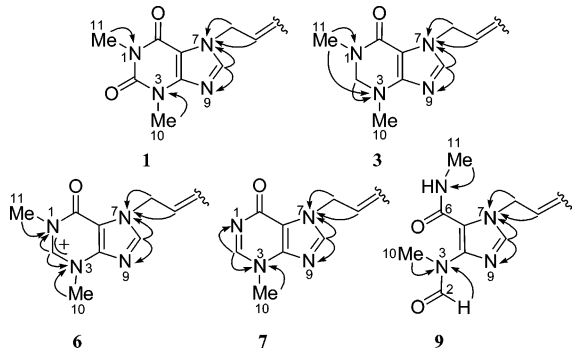


Figure 2. ^{1}H−^{15}N HMBC correlations measured for the different heterocyclic moieties.

the basis of the distinction between the two *N*-methyl groups by the HMBC experiment (Figure 1, as shown for **4**). $^2J_{NH}$ and $^4J_{NH}$ correlations from both H_2-2 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 HRMALDIMS the molecular composition $C_{27}H_{42}N_4O_2$ ($[M + H]^+$, *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 DDQ as the oxidant.[21]

The FAB mass spectrum of the charged molecule (**6**) exhibited a molecular ion $[M + H]^+$ at *m*/*z* 454.3308. The molecular formula $C_{27}H_{41}N_4O_2$ was determined by HRMALDIMS. The ^{1}H 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_2-2 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 bicylic moiety as described in Figure 1. Further support for the structure came from ^{1}H−^{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_2-1′ and H-2′ to N-7 (Figure 2). $^2J_{NH}$ 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. ^{1}H and ^{13}C NMR Spectroscopic Data for Malonganenones D−H (**7**−**11**)[a]

	7		**8**		**9**		**10**		**11**	
position	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

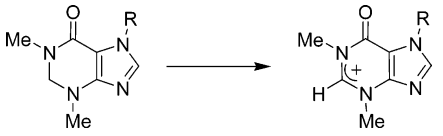
[a] Compounds **7**, **8**, and **11** were measured at 400 MHz in $CDCl_3$; **9** and **10** in d_6-DMSO.

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	233.2	232.3	248.8

[a] ^{15}N chemical shifts deduced from ^{1}H−^{15}N HMBC experiments.

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



4 **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 $[M + Na]^+$ at *m*/*z* 461.2890 The molecular formula was established as $C_{26}H_{38}N_4O_2$ from HRMALDIMS and ^{13}C NMR data, suggesting 10 degrees of unsaturation. The ^{13}C and ^{1}H 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 ^{1}H−^{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. $^2J_{NH}$ 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 $C_{27}H_{43}N_4O_3$ (HRMALDIMS, *m*/*z* 471.3340), indicating nine degrees of unsaturation. The molecular formula together with the NMR spectroscopic data (Table 3) suggested an 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 ^{1}H−^{15}N HMBC experiment of **9** confirmed the trisubstituted imidazole structure by two $^2J_{NH}$ 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). $^2J_{NH}$ correlation observed from Me-11 to its neighbor amide N-atom (N-1), resonating at 103.9, and the residual $^1J_{NH}$ 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 $C_{21}H_{36}NO_2$ ($[M + H]^+$ *m*/*z* 334.2750), indicating five degrees of unsaturation. Careful analysis

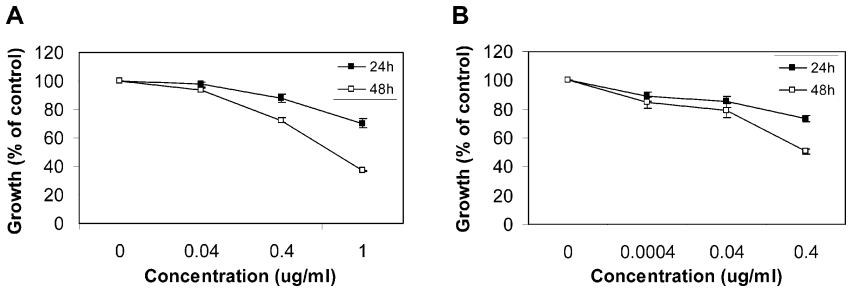


Figure 3. Nuttingins C−E (**3**−**5**) inhibit growth of K562 (A) and UT7 (B) cell lines. Cells were exposed to the indicated amounts of **3**−**5** for 24 and 48 h. Cell growth is expressed as a percentage of the control. Each value represents the mean ± SD of 3 experiments.

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 $\Delta^{11'}$ 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 K562[22] and UT7[23] 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 μg/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 μg/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. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance-400 spectrometer. ^{1}H, ^{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 ^{1}H−^{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 Uvinage, 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 chromotographed 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 × 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), affordeding 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}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRMALDIMS $[M + H]^+$ *m/z* 469.3168 (calcd for $C_{27}H_{41}N_4O_3$, 469.3173).

Nuttingin B (2): colorless oil; IR (CH_2Cl_2) ν_{max} 2959, 1705, 1644, 1548, 1462, 1384, 1218, 1212 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRMALDIMS $[M + H]^+$ *m/z* 469.3170 (calcd for $C_{27}H_{41}N_4O_3$, 469.3173).

Nuttingin C (3): colorless oil; IR (CH_2Cl_3) ν_{max} 2960, 2930, 1659, 1450, 1383, 1214 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRMALDIMS $[M + H]^+$ *m/z* 455.3384 (calcd for $C_{27}H_{42}N_4O_2$, 455.3386).

Nuttingin D (4): colorless oil; IR (CH_2Cl_2) ν_{max} 2960, 1642, 1451, 1383, 1217, 1212 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRMALDIMS $[M + H]^+$ *m/z* 455.3383 (calcd for $C_{27}H_{42}N_4O_2$, 455.3386).

Nuttingin E (5): colorless oil; IR (CH_2Cl_2) ν_{max} 2960, 1644, 1450, 1383, 1218, 1214 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRMALDIMS $[M + H]^+$ *m/z* 455.3383 (calcd for $C_{27}H_{41}N_4O_3$, 455.3386).

Nuttingin F (6): colorless oil; IR (CH_2Cl_2) ν_{max} 2958, 2360, 1728, 1677, 1214, 1138 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRMALDIMS $[M + H]^+$ *m/z* 454.3308 (calcd for $C_{27}H_{41}N_4O_2$, 454.3310).

Malonganenon D (7): colorless oil; IR (CH_2Cl_2) ν_{max} 2960, 1644, 1452, 1384, 1218, 1212, 1030 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 3; HRMALDIMS $[M + Na]^+$ *m/z* 461.2890 (calcd for $C_{26}H_{38}N_4O_2Na$, 461.2887).

Malonganenon E (8): colorless oil; IR (CH_2Cl_2) ν_{max} 2959, 1644, 1462, 1384, 1218, 1212, 1096 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 3; HRMALDIMS $[M + Na]^+$ *m/z* 461.2890 (calcd for $C_{26}H_{38}N_4O_2Na$, 469.2887).

Malonganenon F (9): colorless oil; IR (CH_2Cl_2) ν_{max} 2960, 1665, 1564, 1454, 1213 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; HRMALDIMS $[M + H]^+$ *m/z* 471.3340 (calcd for $C_{27}H_{43}N_4O_3$, 471.3335).

Malonganenon G (10): colorless oil; IR (CH_2Cl_2) ν_{max} 2960, 1665, 1612, 1451, 1358, 1218, 1212 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; HRMALDIMS $[M + H]^+$ *m/z* 471.3340 (calcd for $C_{27}H_{43}N_4O_3$, 471.3335).

Malonganenon H (11): colorless oil; IR (CH_2Cl_2) ν_{max} 3441, 2931, 1682, 1611, 1450, 1384, 1212, 1141 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; HRCIMS $[M + H]^+$ *m/z* 334.2750 (calcd for $C_{21}H_{36}NO_2$, 334.2746).

Cell Culture. The human leukemia cell lines K562[22] and UT7[23] were grown in RPMI 1640 and IMDM medium, respectively, supplemented with 10% fetal bovine serum (FBS), 2 mM l-glutamine, 100 μg/mL streptomycin, and 100 μ/mL penicillin. UT7 cells were cultured in the presence of 2 units/mL erythropoietin (Eprex). Cells were maintained at 37 °C in a 5% CO_2 humidified incubator.

Colorimetric MTT Assay. Cells (4×10^3) 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 μg/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 ^{13}C NMR spectra for compounds **2**, **4**, and **6**, including HMBC and ^{15}N-HMBC for compound **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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