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Frondosins, Five New Sesquiterpene Hydroquinone Derivatives with Novel Skeletons from the Sponge *Dysidea frondosa*: Inhibitors of Interleukin-8 Receptors

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Abstract:. Bioassay-guided fractionation of the EtOAc extract of the sponge *Dysidea frondosa* collected in Pohnpei yielded five sesquiterpenes, frondosins A - E (**1-5**). The structures and relative stereochemistry of the frondosins were established by interpretation of spectral data. Frondosins A - E (**1-5**), which possess novel carbon skeletons, were found to be inhibitors of interleukin-8 receptors and protein kinase C in the low micromolar range. © 1997 Elsevier Science Ltd.

Introduction: Interleukin-8 (IL-8/NAP-1), a chemoattractant for neutrophils, is produced by macrophages, fibroblasts, endothelial and epithelial cells exposed to Tissue Necrosis Factor (TNF) and IL-1 β.[1-4] IL-8 promotes the accumulation and activation of neutrophils and has been implicated in a wide range of acute and chronic inflammatory disorders including psoriasis and rheumatoid arthritis.[5-6] Hence, the IL-8 receptor antagonists represent a promising target for the development of novel anti-inflammatory agents.

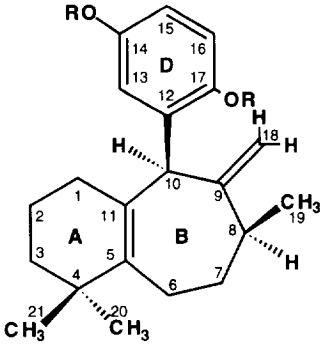
Marine organisms are a rich source of novel compounds with useful pharmacological properties.[7] Sponges have contributed significantly to the array of new structural types derived from marine organisms. As part of our continuing search for biologically active compounds with potential utility in the treatment of inflammatory diseases, we established a high throughput screen to evaluate the ability of natural product extracts to inhibit the binding of IL-8 to its receptors. An extract from one of the sponges collected in Pohnpei, Federated States of Micronesia, showed the ability to inhibit IL-8 receptors and was selected for fractionation. We now report the isolation from *Dysidea frondosa* and the structure determination of five novel sesquiterpenes derivatives, frondosins A - E (**1-5**), which possess novel carbon skeletons and have useful biological activity.

The freeze-dried sponge was extracted sequentially with ethyl acetate and methanol. Bioassay-directed fractionation of the ethyl acetate extract, which was found to inhibit IL-8 receptors, by column chromatography on Sephadex LH-20 followed by silica gel column chromatography yielded several active

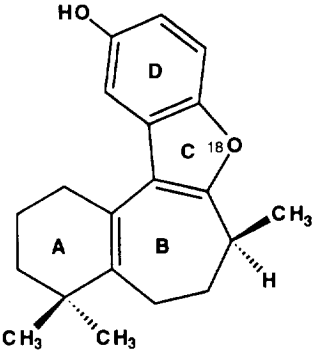
fractions. Further purification of these fractions by RP-18 preparative thin layer chromatography followed by silica gel HPLC led to the isolation of frondosins A - E (**1-5**).

Frondosin A (**1**), $[\alpha]_D = +31.5$, was isolated as a white powder. The presence of phenolic groups was indicated by a positive ferric chloride test. That observation was further supported by preparation of the dimethyl ether (**1a**), diacetate (**1b**) and dibenzoate (**1c**) derivatives of **1**. The low resolution DCI mass spectrum of frondosin A (**1**) exhibited a strong molecular ion at *m/z* 312, with two exchangeable hydrogens, which corresponded to a molecular formula of $C_{21}H_{28}O_2$ and required eight degrees of unsaturation. An intense fragment ion at *m/z* 203 was interpreted as the loss of hydroquinone based on high resolution measurements, leaving a residual C_{15} fragment which contained two rings and two double bonds.

The ^{1}H NMR spectrum (Table 1) of **1** showed an aromatic ABX spin system (between δ6.74 and 6.58) and two broad hydroxyl singlets (δ5.35 and 4.94, which disappeared after the addition of D_2O), typical of a mono-substituted hydroquinone, as well as a pair of exocyclic methylene hydrogens (δ4.83 and 4.50), a narrow methine multiplet (δ3.94), another methine multiplet (δ2.48), five pair of methylene multiplets (between δ2.48 and 1.34), a six-proton methyl singlet (δ1.07) and a methyl doublet (δ1.04, J = 6.9 Hz). The



1 R = H
1a R = CH_3
1b R = $COCH_3$
1c R = COPhBr



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^{13}C GASPE NMR spectrum (GAted SPin Echo pulse sequence which generates a ^{1}H decoupled ^{13}C spectrum with multiplicity information phase encoded) exhibited 21 carbons which included five methine, six

TABLE 1. ^{1}H NMR Assignments for Frondosins A-E (1 - 5) in $CDCl_3$ at 400 HMz

#	1	2	3	4	5
1	1.84 (2H, m)	2.55 (2H, t,*J* =5.9)	2.32 (1H, dt,*J* =16.7,4.5)	2.27 (1H, dt,*J* =4.6,16.7)	2.30 (1H, dt, *J* =4.5,16.7)
			1.91 (1H, m)	1.78 (1H, m)	1.78 (1H, m)
2	1.55 (1H, m)	1.71 (2H, m)	1.65 (2H, m)	1.64 (2H, m)	1.64 (2H, m)
	1.53 (1H, m)				
3	1.51 (2H, m)	1.56 (2H, m)	1.55 (2H, m)	1.58 (2H, m)	1.58 (2H, m)
6	2.48 (1H, m)	2.15 (1H, m)	2.02 (1H, m)	2.01 (1H, m)	2.01 (1H, m)
	2.01 (1H, ddd, *J*=4.2,4.2,14.6)	2.10 (1H, m)	1.75 (1H, m)	1.85 (1H, m)	1.85 (1H, m)
7	1.87 (1H, m)	2.12 (1H, m)	2.19 (1H, m)	2.06 (1H, m)	2.06 (1H, m)
	1.34 (1H, m)	1.62 (1H, m)	1.79 (1H, m)	1.81 (1H, m)	1.81 (1H, m)
8	2.48 (1H, m)	3.19 (1H, m)	2.72 (1H, m)	2.66 (1H, m)	2.66 (1H, m)
10	3.94 (1H, dd, *J* =1.1,1.1)				
13	6.58 (1H, d, *J* =3.0)	7.12 (1H,d,*J* =2.5)	5.86 (1H, d,*J* =1.7)	5.70 (1H, d,*J* =2.0)	5.79 (1H, d,*J* =2.0)
15	6.64 (1H, dd,*J* =3.0,8.6)	6.71 (1H, dd,*J* =2.5,8.7)	6.12 (1H, dd, *J* =1.7,9.8)	6.18 (1H, dd,*J* =2.0,10.0)	6.31 (1H, dd,*J* =2.0,10.2)
16	6.74 (1H, d, *J* =8.6)	7.24 (1H, d,*J* =8.7)	7.07 (1H, d,*J* =9.8)	6.75 (1H, d,*J* =10.0)	6.70 (1H, d,*J* =10.2)
18	4.83 (1H, dd, *J* =0.8,1.1)		2.79 (1H, dd,*J* =2.0,16.7)	4.69 (1H, dd, *J* =1.0,18.6)	4.62 (1H, dd,*J* =1.0,18.3)
	4.50 (1H, dd, *J* =0.8,1.1)		2.73 (1H, dd,*J* =1.2,16.7)	4.48 (1H, dd,*J* =1.0,18.6)	4.45 (1H, dd,*J* =1.0,18.3)
19	1.04 (3H, d, *J* =6.9)	1.35 (3H, d, *J* =7.0)	1.11 (3H, d,*J* =6.9)	1.03 (3H, d, *J* = 7.0)	1.04 (3H, d,*J* =7.0)
20	1.07 (3H , s)	1.09 (3H , s)*	1.13 (3H, s)	1.12 (3H, s)	1.12 (3H, s)
21	1.07 (3H , s)	1.10 (3H , s)*	1.04 (3H, s)	1.04 (3H, s)	1.04 (3H, s)
OH	5.35 (1H, OH-17)	4.77 (1H, OH-14)	2.13 (1H, OH-17)	3.66 (1H, OH-17)	3.24 (3H, OMe-22)
	4.94 (1H, OH-14)				

* These chemical shift assignments are interchangeable.

TABLE 2. ^{13}C NMR Assignments for Frondosins A-E (1 - 5) in $CDCL_3$ at 100 HMz

C	1	2	3	4	5
1	32.0	30.5	27.5	27.5	27.7
2	19.7	20.0	19.3	19.6	19.6
3	39.3	39.5	39.3	39.5	39.5
4	35.6	35.7	35.3	35.1	35.1
5	140.4	144.3	148.1	147.5	147.1
6	25.3	26.0	26.3	25.9	25.9
7	37.6	38.5	45.4	44.9	44.9
8	36.9	34.7	33.4	33.8	33.8
9	155.2	160.2	161.0	145.9	145.6
10	56.2	116.3	138.3	130.5	130.3
11	129.4	123.8	125.9	126.4	126.4
12	128.3	129.6	167.6	147.3	146.9
13	116.5	107.3	116.3	118.8	120.6
14	149.4	150.7	186.5	186.5	186.3
15	114.6	111.1	129.6	128.3	130.5
16	117.1	110.9	145.9	144.1	142.1
17	149.0	149.1	72.7	86.7	90.3
18	107.7		43.0	61.4	61.9
19	20.0	19.7	17.7	15.1	15.1
20	27.9*	28.9*	29.7	29.6	29.6
21	27.7*	27.9*	27.8	28.4	28.4
22					51.0

* These chemical shift assignments are interchangeable.

methylene, three methyl and seven quaternary carbon signals (Table 2). The combination of the NMR spectral features confirmed the presence of a dihydroquinone moiety (proposed by mass spectral data) in frondosin A (**1**) accounting for six of the carbon signals. Since three of the remaining fifteen carbons were attributable to methyl signals and one to an exocyclic methylene group, the lack of an aliphatic chain further suggested that the remaining eleven-carbon bicyclic moiety consisted of a six-membered ring fused to a seven-membered ring.

As confirmation of this proposal the two doublet of doublets at δ4.83 and 4.50 (H-18) of the exocyclic methylene showed COSY correlations with each other and H-10 and displayed HMBC correlations with ring B carbons C-8, C-9 and C-10. The H-10 methine showed an additional benzylic coupling with H-13 of ring D indicating that the hydroquinone ring was connected to C-10, while the H-8 multiplet was coupled to the CH_3-19 methyl doublet (δ1.04) and the H-7 methylene protons at δ1.87 and δ1.34. The H-7 protons further correlated to the H-6 methylenes at δ2.48 and δ2.01. As further proof of its centralized position, H-10 showed HMBC correlations with C-12, C-13 and C-17 in hydroquinone ring D, with quaternary olefins C-5 and C-11 in ring B and with methylene C-1 in ring A. Thus the remaining double bond was situated between the ring junction carbons, C-5 and C-11, adjacent to H-10. On the opposite side of ring B, the H-6 methylene hydrogens showed correlations to C-5, C-7, C-8 and C-11, clearly indicating that ring B was seven-membered, and to the aliphatic quaternary carbon C-4 in ring A. The protons of two geminal methyls, CH_3-20 and CH_3-21, correlated to each other's carbons (C-20 and C-21), C-4, C-5 and the aliphatic C-3 methylene. Finally, the COSY data showed correlations between the H-1 protons at δ1.84, H-2 at δ1.55 and δ1.53 and H-3 at δ1.51, thus firmly establishing the structure of frondosin A (**1**).

The relative stereochemistry of frondosin A (**1**) was established by nOe difference data which is represented by arrows about an energy minimized model of **1** in Figure 1 below. Saturation of the axial H-10 caused nOe enhancements of H-13 on the phenyl ring and the axial H-8 establishing that both H-10 and H-8 were situated on the same face of ring B. Irradiation of H-8 caused an enhancement of CH_3-19, which in turn affected H-18 (δ4.83) upon saturation. The equatorial H-6 doublet of doublets of doublets at δ2.01 shared an nOe with the combined CH_3-20/21 singlet at δ1.07. The broad phenol resonance at δ4.94 shared an nOe with

H-13 indicating that it was attached at the adjacent C-14 position. The absolute stereochemistry was not determined.

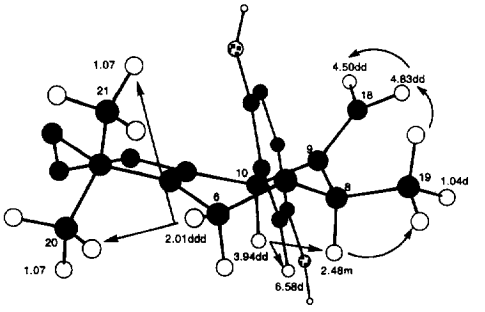


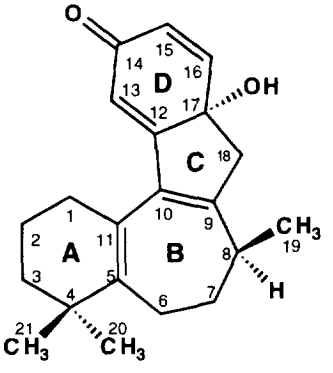
Figure 1. Molecular model of the frondosin A (**1**) with arrows representing nOe enhancements.

Frondosin B (**2**), $[\alpha]_D = +18.6°$, which was isolated as a colorless gum, displayed a molecular ion at *m/z* 296 with one exchangeable hydrogen. The molecular formula was determined to be $C_{20}H_{24}O_2$ by HRDCIMS, one carbon and four hydrogens less than frondosin A (**1**), indicating the presence of nine double bond equivalents. The IR spectrum was similar to that of **1**. The ^{1}H NMR spectrum of **2** had an ABX system between δ7.24 and δ6.71 similar to **1**, but the exocyclic H-18 methylene signals and the H-10 methine signal observed at δ3.94 in **1** were absent in **2**. It also exhibited a broad hydroxyl singlet (δ4.77), a methine multiplet (δ3.19), five pair of methylene multiplets (between δ2.55 and 1.56), two methyl singlets (δ1.10 and 1.09) and a methyl doublet (δ1.35). Together the mass spectral and ^{1}H NMR data for **2** suggested that the C-9/18 double bond in **1** had been oxidatively cleaved to give a ketone that then underwent ring closure with the phenolic hydroxyl to give a furan. The ^{13}C GASPE spectrum of **2** had 20 carbon resonances. The absence of an olefinic exocyclic methylene carbon signal and the presence of signals at δ116.3 and δ160.2 supported the proposed benzofuran ring system.

The long-range correlations observed in the HMBC spectrum of **2** for rings A and B were analogous to those observed for **1**, and the aromatic ABX protons, H-13 through H-16, showed similar correlations within aromatic ring D. The CH_3-19 doublet on ring B showed HMBC correlations with C-7, C-8 and the C-9 quaternary carbon (δ160.2). In similar fashion, the H-1 methylene signals in ring A correlated to methylenes C-2 and C-3 and quaternary carbon signals C-5, C-10 and C-11. Collectively these correlations described a

tetra-substituted C-9/10 double bond in ring B with the chemical shift of C-9 at δ160.2 indicating attachment of oxygen. As expected, H-13 and H-15 in ring D correlated to each other's carbon as well as to C-17 and the C-14 phenolic carbon. The chemical shifts of C-14 and C-17, two para-positioned quaternary carbons, indicated that they were both oxygenated. The H-16 signal also correlated to C-14 and C-12. Since all of the atoms in the molecular formula were already accounted for, these carbon chemical shifts implied that C-12 was connected to C-10 and that C-17 was connected to C-9 in ring B through an oxygen bridge forming a ring C furan. This argument was supported by a strong nOe observed between the H-13 doublet in ring D and the H-1 methylene protons in ring A.

Frondosin C (**3**), $[\alpha]_D$ = +9.4°, obtained as an oil, showed a molecular ion at *m/z* 310, two Daltons lower than **1**, with one exchangeable hydrogen, and had fragment ions at *m/z* 292 and *m/z* 282 attributable to the losses of H_2O and CO. The HRDCIMS suggested the molecular formula to be $C_{21}H_{26}O_2$. The IR spectrum of **3** showed strong bands for a hydroxyl (3357 cm^{-1}) and an α,β-unsaturated carbonyl (1668 cm^{-1}), and its ^1H NMR spectrum included an ABX spin system at δ7.07 (d, 1H, *J* = 9.8 Hz), δ6.12 (dd, 1H, *J* = 9.8, 1.7 Hz) and δ5.86 (d, 1H, *J* = 1.7 Hz). These coupling constants pointed to a quinonoid structure in ring D. The aliphatic region of the proton spectrum contained two slightly non-equivalent geminal signals at δ2.79 and 2.73, a methine at δ2.72, a broad hydroxyl resonance at δ2.13, five pair of methylene multiplets between δ2.32 and 1.55 and three methyl signals, two singlets at δ1.13 and 1.04 and a doublet at δ1.11. The



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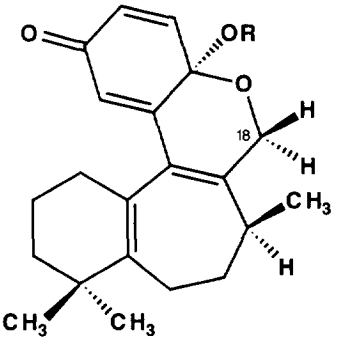

4 R = OH
5 R = OCH_3

MS and IR data together with the ^{1}H NMR data of **3** suggested that ring D was quinonoid and that ring C contained a cyclopentene moiety with a tertiary hydroxyl group. The ^{13}C GASPE spectrum of **3** displayed 21 carbons of which there were eight quaternary, four methine, six methylene, and three methyl carbon signals. A carbonyl signal at δ186.5 supported the presence of a quinonoid moiety. HMQC and HMBC correlations indicated that the ring A and B resonances were similar to their counterparts in **1** and **2**. Long range correlations between the H-18 methylene protons in ring C and C-9 and C-10 in ring B indicated that they were situated adjacent to the C-9/10 double bond. The H-18 methylenes also displayed correlations with C-12 and C-17 in ring D. The H-16 signal correlated with the quaternary carbon C-12 and the carbonyl at C-14. The H-13 doublet correlated with C-15 and C-17 in ring D as well as C-10 in ring B. The δ72.7 chemical shift of the quaternary C-17 clearly established that it was an oxygenated aliphatic carbon and was therefore the site of attachment for the hydroxyl group.

The relative stereochemistry of **3** was established by coupling constants, COSY and HMBC correlations, and nOe difference data. Saturation of the H-13 resonance at δ5.86 enhanced the H-1_{eq} methylene signal at δ2.32 establishing that the relationship between rings A and D was conserved from compound **2**. Irradiation of H-7β (δ1.79) caused an enhancement of the H-18 methylene signal at δ2.73 while saturation of CH_3-19 enhanced both H-18 protons. Thus, the H-18 signal at δ2.73 occupied the β position while the H-18 signal at δ2.79 occupied the α position. The hydroxyl group at C-17 was assigned to the α face because the hydroxyl proton shared an nOe enhancement with H-18α.

Frondosin D (**4**) was isolated as a colorless solid, $[\alpha]_D$ +29.6°, which had a molecular weight of 326 Daltons by DCIMS, one oxygen more than **3**, with one exchangeable hydrogen. A fragment ion at *m/z* 308 was derived from the loss of water from the molecular ion. The molecular formula of $C_{21}H_{26}O_3$ was deduced from the HRDCIMS. The UV and IR spectra of **4** were almost identical to those of **3**, and the ^{1}H NMR spectrum of **4** closely resembled that of **3** except that the H-18 methylene signals had shifted noticeably downfield from near δ2.75 in **3** to approximately δ4.55 in **4**. The ^{13}C GASPE NMR spectrum of **4** contained 21 carbons and was similar to that of **3** with the exceptions of the 18 ppm downfield shift of the now oxygenated C-18 signal and the 14 ppm downfield shift of the now doubly oxygenated C-17. Based on this data it appeared that ring C in **4** was expanded to a six-membered pyran ring.

The presence of the quinonoid carbonyl signal at δ186.5 and the correlations involving the ring A and B resonances indicated that these regions in frondosin D (**4**) were similar to their counterparts in **3**. Long-range correlations between the H-18 protons in ring C and C-9 and C-10 in ring B indicated that they were still situated adjacent to the C-9/10 double bond. The H-18 methylene signal centered at δ4.48 also displayed a correlation with C-17 establishing the presence of an ether oxygen between C-17 and C-18 in ring C. The chemical shift of C-17 (δ86.7) was characteristic of a hemiketal carbon.

The relative stereochemistry of **4** was proposed to be the same as that of **3** based on similarities in chemical shifts, proton-proton coupling constants, COSY and HMBC correlations, and nOe difference data. The H-8 signal shared a transperiplanar coupling of approximately 13 Hz with H-7_{ax} indicating that the ring B conformation was similar to that in **3**. Irradiation of H-8 at δ2.66 caused small enhancements of H-7α and H-18α (δ4.69), and saturation of CH_3-19 enhanced both H-18 signals. Therefore H-8 and H-18 (at δ4.69) protons were situated below the face of ring C. The stereochemistry of the C-17 hydroxyl group was difficult to determine solely by nOe data. However, after the C-17 methoxyl group in analog **5** was ascertained to be below the plane of ring C, a comparison of the chemical shifts of the non-equivalent H-18 hydrogens in compounds **4** and **5** clearly indicated that the C-17 hydroxyl group in **4** was also situated below the plane of ring C.

Frondosin E (**5**) was obtained as a clear oil, $[\alpha]_D$ +26.1°, and its DCIMS displayed a molecular ion and base peak at *m/z* 340, fourteen Daltons higher than **4**, corresponding to a molecular formula of $C_{22}H_{28}O_3$. The UV, IR, ^1H and ^{13}C NMR spectra of **5** were identical to those of **4** except for a three proton methoxyl singlet at δ3.24 and a methoxyl carbon signal at δ51.0. Mass spectral data indicated that the frondosin E (**5**) was the methyl ether of **4** as reflected by the loss of methanol from the parent ion of **5** rather than water as observed for **4**. The coupling constants and nOe enhancements of all protons in frondosin E (**5**) were identical to those in **4** implying similar relative stereochemistry. Since frondosin (**5**) adopted a bent "u" shape, there was a bay region between rings A and D and as a result H-13 on the quinone ring shared a strong nOe with H-1_{eq}. Irradiation of the methoxyl singlet at δ3.24 produced an nOe with the H-18α proton at δ4.62 and with H-16. Therefore the methyl ether was situated on the α face of ring C.

Table 3. Bioactivity of Frondosins A-E (1-5)

	IC_{50} (μM)		
Compound	**IL-8 Rα**	**IL-8 Rβ**	**PKC-α**
Frondosin A	3.4	3.2	1.8
Frondosin B	9.6	10.8	4.8
Frondosin C	84	23.6	20.9
Frondosin D	98	10.8	26
Frondosin E	64	37.1	30.6

The IL-8 receptor and protein kinase C (PKC) activities of frondosins A - E (**1-5**) are presented in Table 3 which indicated similar potencies across all three targets. Frondosin A (**1**) was found to be the most potent compound against both IL-8 Rα and IL-8 Rβ receptors with IC_{50} values of 3.4 and 3.2 μM and also displayed potent activity against PKC-α (IC_{50} =1.8 μM). The dimethyl ether (**1a**), the diacetate (**1b**) and the dibenzoate (**1c**) of frondosin A showed weak or no activity in the bioassay which suggested that the free hydroxyl groups were essential for activity in frondosin A (**1**). From the biological data presented in Table 3, it appears that the activity is non-selective which may indicate that these compounds are reactive rather than representing a true agonist/antagonist event.

Experimental Section

General: The IR spectra were recorded on a Nicolet Model 20 DXB FTIR spectrometer. All homonuclear and heteronuclear one- and two-dimensional NMR data were recorded on a Bruker AMX-400 spectrometer in CD_3OD. The LRDCI mass spectra were acquired on a Finnigan 4610 quadrupole mass spectrometer, and HRDCI mass spectra were acquired on a VG-70SE mass spectrometer. Analytical and preparative TLC were carried out on precoated Si gel G (Kiesel gel G254) and reversed-phase (Whatman KC18F) plates. The UV spectra were recorded on a Beckman DV-7 spectrophotometer. Optical rotations were recorded on Perkin-Elmer 241 MC polarimeter. Reagent grade chemicals (Fisher and Baker) were used throughout.

Materials and Methods: [^{125}I] IL-8 (human recombinant) was obtained from Amersham Corp., Arlington Heights, IL, with specific activity 2000 Ci/mmol. All other chemicals were of analytical grade. High levels of recombinant human IL-8 type α and β receptors were individually expressed in Chinese Hamster ovary cells as described previously.[9]

IL-8 Receptor Binding Assays: The Chinese hamster ovary membranes were homogenized according to a previously described protocol.[8] Membrane protein concentration was determined using Pierce Co. micro-assay kit using bovine serum albumin as a standard. All assays were performed in a 96-well microplate format. Each reaction mixture contained [^{125}I] IL-8 (0.25 nM), 0.5 μg/mL of IL-8Rα or 1.0 μg/mL of IL-8Rβ membranes in 20 mM bistrispropane and 0.4 mM Tris HCl buffers, pH 8.0, containing 1.2 mM $MgSO_4$, 0.1 mM EDTA, 25 mM NaCl and 0.03% CHAPS peptide at relevant concentrations. The assay was initiated by addition of [^{125}I] IL-8. After 1 hr at room temperature the plate was harvested using a Tomtec 96-well harvester onto a glass-fiber filtermat blocked with 1% polyethylenimine/0.5% BSA and washed three times with 25 mM NaCl, 10 mM Tris HCl, 1 mM $MgSO_4$, 0.5 mM EDTA, 0.03% CHAPS, pH 7.4. The filter was then dried and counted on the Betaplate liquid scintillation counter.

Collection, Extraction and Isolation: The sponge, voucher number POH93-084, was collected on February 4, 1993, from a depth of 12-21 m at Nan Madol, Pohnpei, Federated States of Micronesia, and the specimen were frozen immediately and kept at -20°C until extraction. This sponge forms erect, compressible fingers arising from an encrusting base. The surface is highly conulose with a delicate surface tracery, and the overall construction is cavernous. The color is pinkish purple with a tan interior. The skeleton consists of fibers packed with spicule debris that form a relatively regular mesh. The sample is most closely comparable to *Dysidea frondosa* Bergquist (Family Dysideidae, Order Dictyoceratida) as described by Bergquist.[10] A voucher specimen has been deposited at the Natural History Museum, London, United Kingdom (BMNH 1995:10:4:5). The freeze-dried sponge (78 g) was extracted with ethyl acetate and methanol to give 3.1 and 8.9 g extracts, respectively. The pale yellow ethyl acetate (3 g) extract which showed IL-8 receptor inhibitory activity was applied to a column of Sephadex LH-20 and eluted with methanol:methylene dichloride:hexane (1:1:1). The IL-8 active fractions were monitored by bioassay and pooled. Combined active fractions (212

mg) were chromatographed over a column of silica gel eluting with acetone:hexane (25:75). Several 15 mL fractions were collected and monitored by TLC. Like fractions were combined to give four individual fractions (A-D). RP-18 preparative thin layer chromatography of the IL-8 active fraction B (74 mg) using H_2O:CH_3CN (15:85) followed by silica gel HPLC (monitored by refractive index detector) employing ethyl acetate:hexane (30:70) as the solvent system, afforded frondosins A (**1**, 37 mg), B (**2**, 10.6 mg), C (**3**, 6.5 mg), D (**4**, 5.2 mg) and E (**5**, 7.1 mg) in pure form.

Frondosin A (**1**): White powder, m.p. 111-113° C, $[\alpha]_D$ +31.5° (*c* = 0.25, MeOH); UV (MeOH) λ_{max} 232 and 296 nm; IR (KBr) ν_{max} 3380-3320, 3000-3100, 2800-3100, 1653, 1637, 1495, 1383, 1383, 1360, 889 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; LRDCIMS *m/z* 312; HRDCIMS (methane) calcd for $C_{21}H_{28}O_2$ *m/z* 312.2089, found *m/z* 312.2087.

Preparation of frondosin A dimethyl ether (**1a**): A solution of **1** (5 mg) and methyl iodide (0.5 ml) in dry acetone (15 ml) containing anhydrous potassium carbonate (3 g) refluxed at 70° C for 12 h. The mixture was filtered, and the solvent was evaporated under vacuum. The residue was purified by silica gel PTLC to afford **1a** (4.5 mg) as an oil. ^1H NMR ($CDCl_3$) δ6.85 (1H, d, *J* = 8.4 Hz), 6.75 (1H, dd, *J* = 2.9, 8.4 Hz), 6.71 (1H, d, *J* = 2.9 Hz), 4.65 and 4.25 (1H each, dd, *J* = 0.8, 1.1 Hz), 4.13 (1H, dd, *J* = 1.1, 1. 1 Hz), 3.79 (3H, s), 3.75 (3H, s), 2.50 (1H, m), 1.85-2.01 (m, 3H), 1.21-1.71 (7H, m), 1.11 (3H, d, *J* = 7 Hz), 1.08 (3H, s), 1.07 (3H, s); LRDCIMS *m/z* 340.

Preparation of frondosin A diacetate (**1b**): A solution of **1** (5 mg) in acetic anhydride (0.5 ml) and pyridine (0.1 ml) was left overnight at room temperature, then evaporated under vacuum. The residue was purified by silica gel PTLC to yield **1b** (5 mg) as a colorless gum. ^1H NMR ($CDCl_3$) δ7.09 (1H, d, *J* = 8.5 Hz), 7.03 (1H, dd, *J* = 3.0, 8.5 Hz), 7.00 (1H, d, *J* =3.0 Hz), 4.71 and 4.21 (1H each, dd, *J* = 0.8, 1.1 Hz), 3.96 (1H, dd, *J* = 1.1, 1.1 Hz), 2.45 (1H, m), 2.28 (3H, s), 2.25 (3H, s), 1.20-2.01 (10H, m), 1.05 (3H, d, *J* = 6.9 Hz), 1.05 (3H, s), 1.04 (3H, s); LRDCIMS *m/z* 396.

Benzoylation of Frondosin A (**1**): To a solution of frondosin A (10 mg) in acetonitrile (1.5 ml) was added 4-(*N*,*N*-dimethylamino)pyridine (5 mg) and 4-bromobenzoyl chloride (30 mg). The resulting white

suspension was stirred at room temperature for 3 h. The reaction mixture was diluted with cold 0.1 N HCl and extracted with ether (2 x 25 ml). The ether layer was washed successively with cold 0.1 N HCl (twice), saturated aqueous $NaHCO_3$, water, brine and then dried over $MgSO_4$. Evaporation of the solvent afforded a residue that was purified by silica gel PTLC with acetone:hexane (20:80) to yield **1c** as an amorphous powder (13 mg). ^{1}H NMR ($CDCl_3$) δ8.07 (2H, d, J = 8.4 Hz), 8.00 (2H, d, J = 8.4 Hz), 7.91 (2H, d, J = 8.4 Hz), 7.66 (2H, d, J =8.4 Hz), 7.27 (1H, d, J = 8.4 Hz), 7.18 (1H,dd, J = 3.0, 8.4 Hz), 7.11 (1H, d, J = 3.0 Hz), 4.75 and 4.32 (1H each, dd, J = 0.8, 1.1 Hz), 4.01 (1H, dd, J = 1.1, 1.1 Hz), 2.49 (1H, m), 1.20-2.05 (10H, m), 1.03 (3H, d, J = 7.0 Hz), 1.01 (3H, s), 0.99 (3H, s); LRDCIMS *m/z* 676.

Frondosin B (**2**): Colorless gum, $[\alpha]_D$ +18.6° (*c* = 0.17, MeOH); UV (MeOH) λ_{max} 228, 245 and 258 nm; IR (KBr) ν_{max} 3370, 3000-3100, 2800-3000, 1619, 1590, 1462, 1380, 1197 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; LRDCIMS *m/z* 296; HRDCIMS (methane) calcd for $C_{20}H_{24}O_2$ *m/z* 296.1776, found *m/z* 296.1768.

Frondosin C (**3**): Colorless oil, $[\alpha]_D$ +9.4° (*c* = 0.12, MeOH); UV (MeOH) λ_{max} 230, 245 and 295 nm; IR (KBr) ν_{max} 3357, 3000-3100, 2800-3000, 1668, 1605, 1457, 1063 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; LRDCIMS *m/z* 310; HRDCIMS (methane) calcd for $C_{21}H_{26}O_2$ *m/z* 310.2011, found *m/z* 310.2000.

Frondosin D (**4**): Colorless solid, $[\alpha]_D$ +29.6° (*c* = 0.2, MeOH); UV (MeOH) λ_{max} 220, 245 and 321 nm; IR (KBr) ν_{max} 3359, 3000-3100, 2800-3000, 1661, 1619, 1594, 1491 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; LRDCIMS *m/z* 326; HRDCIMS (methane) calcd for $C_{21}H_{26}O_3$ *m/z* 326.1960, found *m/z* 326.1945.

Frondosin E (**5**): Clear oil, $[\alpha]_D$ +26.1° (*c* = 0.09, MeOH); UV (MeOH) λ_{max} 228, 247 and 299 nm; IR (KBr) ν_{max} 3400, 3000-3100, 2800-3000, 1667, 1495, 1457 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; LRDCIMS *m/z* 340; HRDCIMS (methane) calcd for $C_{22}H_{28}O_3$ *m/z* 340.2111, found *m/z* 340.2104.

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