New Sesquiterpene Derivatives from the Sponge *Dysidea* Species with a Selective Inhibitor Profile against Human Phospholipase A_2 and Other Leukocyte Functions

Clelia Giannini,[†] Cécile Debitus,[‡] Rut Lucas,[§] Amalia Ubeda,[§] Miguel Payá,[§] John N. A. Hooper,[⊥] and Maria Valeria D'Auria*,[†]

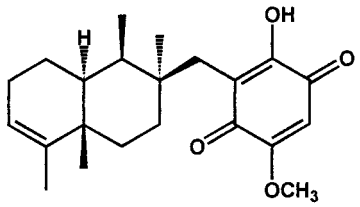
Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II", Via D. Montesano 49, 80131, Naples, Italy, IRD (ex ORSTOM), Centre de Nouméa, BP A5 98848 Nouméa Cedex, New Caledonia, Departamento de Farmacología, Universidad de Valencia, Av. V. Andrés Estelles s/n 46100, Burjassot, Valencia, Spain, and Queensland Centre for Biodiversity, Queensland Museum, P.O. Box 3300, South Brisbane, QLD, 4101, Australia

Received December 29, 2000

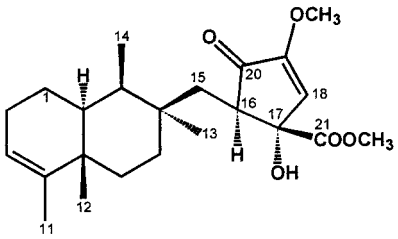
Two new sesquiterpene cyclopentenones, dysidenones A and B (**2**, **3**), and a new sesquiterpene aminoquinone, dysidine (**4**), all containing the same rearranged drimane skeleton, have been isolated from a *Dysidea* sp. sponge, along with bolinaquinone (**1**). The structures were established from 2D NMR data. Bolinaquinone (**1**), dysidine (**4**), and a 1:1 mixture of dysidenones A and B (**2**, **3**) significantly inhibited human synovial phospholipase A_2 (PLA_2) at 10 μM. Compound **4**, which shows an IC_{50} value of 2.0 μM, exerts a higher potency and selectivity toward this enzyme than the reference inhibitor manoalide. In addition, all of these compounds modulated at 10 μM other human leukocyte functions such as the degranulation process measured as elastase release and the superoxide production measured by chemiluminescence.

Recently, from a *Dysidea* sp. sponge collected at the Vanuatu Islands, we isolated a bioactive sesquiterpenoid, dysidotronic acid, which represents the second example of a sesquiterpene derivative with a further rearranged drimane skeleton.[1] It was proved to be a potent and selective human synovial phospholipase A_2 (PLA_2) inhibitor.[1] It is interesting to note that PLA_2 has been recognized to be involved in a wide number of pathophysiological situations, ranging from systemic and acute inflammatory conditions to cancer.[2] Further investigation of this *Dysidea* sp. led to the isolation of three more related minor metabolites, dysidenones A and B (**2**, **3**) and dysidine (**4**), which are the subject of this paper. The new sesquiterpenoid derivatives share the same rearranged drimane skeleton of the previously reported bolinaquinone (**1**),[3] which is the predominant metabolite of the sponge, whereas they differ in the "benzenoid" part attached to the sesquiterpene moiety.

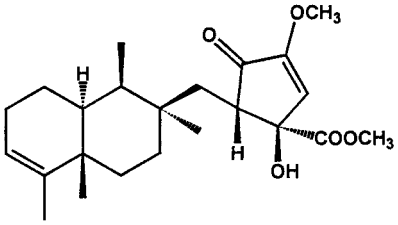
Herein we report the isolation, structure elucidation, and in vitro pharmacological evaluation of **1**−**4** on the inhibition of four different secretory PLA_2s ($sPLA_2$), belonging to groups I (*Naja naja* venom and porcine pancreatic enzymes), II (human synovial recombinant enzyme), and III (bee venom enzyme), as well as on cytosolic PLA_2 ($cPLA_2$), from macrophage line RAW 264.7 (group IV), using manoalide as reference inhibitor. In addition, these compounds were able to modulate other human leukocyte functions such as the degranulation process measured as elastase release and the superoxide production measured by chemiluminescence.



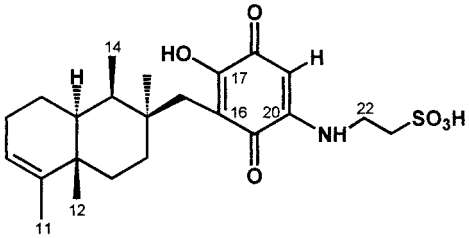
1



2



3



4

Results and Discussion

The lyophilized sponge *Dysidea* sp. was extracted with MeOH, and the combined extracts were fractionated according to the Kupchan partitioning procedure.[4] The chloroform extract was chromatographed by silica gel medium-pressure liquid chromatography (MPLC) (MeOH/

* To whom correspondence should be addressed. Tel: +39-81/678527. Fax: +39-81/678552. E-mail: madauria@cds.unina.it.
[†] Università degli Studi di Napoli "Federico II".
[‡] IRD.
[§] Universidad de Valencia.
[⊥] Queensland Centre for Biodiversity.

10.1021/np000637w CCC: $20.00
Published on Web 04/28/2001

Table 1. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Data [δ ppm, (mult.) J Hz] with HMBC Correlations of Dysidenones A and B (**2**, **3**)

	2		**3**		
position	^{13}C $CDCl_3$	^{1}H $CDCl_3$	^{13}C $CDCl_3$	^{1}H $CDCl_3$	HMBC
1	24.5	1.70−1.15 (m)	24.6	1.70−1.15 (m)	C2
2	26.7	2.04 (m)	26.7	2.04 (m)	
3	120.5	5.14 (br s)	120.6	5.14 (br s)	
4	143.7		143.8		
5	36.8		36.8		
6	31.9	1.47−1.35 (m)	31.9	1.47−1.35 (m)	C5
7	29.5	1.58−1.08 (m)	29.0	1.58−1.08 (m)	
8	35.3		35.9		
9	42.8	1.38 (m)	43.2	1.32 (m)	
10	40.5	1.82 (m)	40.5	1.82 (m)	C2, C5
11	17.7	1.56 (s)	17.7	1.56 (s)	C3, C4, C5
12	19.8	0.92 (s)	19.8	0.93 (s)	C4, C5, C10
13	23.8	1.01 (s)	23.9	1.02 (s)	C7, C8, C9, C15
14	11.6	0.82 (d) 7.0	11.8	0.86 (d) 7.0	C8, C9, C10
15	37.6	2.05−0.93	36.8	1.80−0.98	C7, C8, C9, C13, C17, C20
16	54.9	2.65 (t) 3.2	54.6	2.66 (t) 3.6	C17, C20
17	80.5		80.5		
18	122.3	6.01 (s)	121.5	5.96 (s)	C16, C20
19	158.9		158.7		
20	199.8		199.6		
21	174.6		174.8		
22	53.5	3.75 (s)	53.5	3.74 (s)	C21
OCH_3	57.3	3.75 (s)	57.2	3.76 (s)	C19

CH_2Cl_2, 0−1%) followed by reversed-phase HPLC (20% aqueous MeOH, 0.1% TFA) to give an inseparable mixture of dysidenones A and B (**2** and **3**, 7.8×10^{-4} % yield, dry weight).

More polar dysidine (**4**, 2.1×10^{-2}% yield, dry weight) was obtained, along with known xestoquinol sulfate,[5] from the butanol extract, fractionated by Sephadex LH-20 (eluent MeOH).

Dysidenones A and B were obtained as a mixture. Attempts to separate these compounds by HPLC led to fractions enriched in one component with respect to the other, in such a way that the NMR chemical shifts of each compound could be independently determined. However, we were unable to completely separate the two compounds; thus we performed the spectral analysis on the mixture.

HRFABS data indicated that the two compounds were isomers with the molecular formula $C_{23}H_{34}O_5$ [$(M + H)^+$: 391.2462, calcd for $C_{23}H_{35}O_5$, 391.2484]. ^{1}H and ^{13}C NMR data of the two compounds were very similar, suggesting that they are stereoisomers.

Analysis of NMR data, including 2D COSY, HMQC, and HMBC allowed us to establish the presence in both compounds of the same rearranged drimane skeleton found in bolinaquinone (**1**) and dysidotronic acid. Also, a ROESY experiment, which revealed dipolar coupling between Me-12 and Me-14 and from Me-14 to H_2-15, confirmed the stereochemical analogy for the terpenoid portion of **2** and **3** in comparison to that of **1**.

Taking as an example dysidenone A, the C-15 methylene protons resonate at very different chemical shift values (δ_H 0.93 and 2.05, δ_C 37.6), indicating the proximity to a group with a strong anisotropic effect. In the COSY spectrum both C-15 methylene protons showed a correlation with a methine proton signal at δ 2.65 (overlapped).

In the HMBC spectrum H_2-15 and H-16 showed long-range correlations with a carbonyl group at δ_C 199.8, thus placing a carbonyl functionality at the adjacent position. ^{13}C NMR spectra contained signals ascribable to a trisubstituted double bond (δ_C 158.9, s, and 122.3, d), to an acyl carbonyl group (δ_C 174.6), to two methoxy groups (δ_C 57.3, q, and 53.5, q), and to a quaternary oxygen-bearing carbon atom (δ_C 80.5). The substituted cyclopentenone ring, as depicted in **2**, was assumed on the basis of chemical shift considerations and HMBC data shown in Table 1. The same cyclopentenone portion was found in the diastereomeric dactylospongenones A−D, isolated from the sponge *Dactylospongia* sp.[6] The structure, including relative stereochemistry, of the major dactylospongenone A, was secured by X-ray diffraction analysis. The relative stereochemistry of the remaining three derivatives was assigned on the basis of comparison of spectral data. Spectral data of the cyclopentenone portion of dysidenones A and B match those of dactylospongenones, thus confirming the proposed structure.

The stereochemical relationship between dysidenones A and B could be assigned on the basis of comparison of their spectral data with those of dactylospongenones A−D. It was observed[6] that the relative configuration at C16 and C17 could be established on the basis of the chemical shifts of the H-16, OCH_3 (22). Small but significative differences would also be observed for all carbons belonging to the cyclopentanone system. Both dysidenones A and B display chemical shift values for the aforementioned nuclei very similar to those observed for dactylospongenones A and B, for which a *syn* relationship between H-16 and the hydroxy group at C17 was assigned, and far from those of dactylospongenones C and D, for which an *anti* relationship between H-16 and the hydroxy group at C17 was assigned.

In dactylospongenones the stereochemistry at C16 relative to the bicyclic system was assigned on the basis of the chemical shift value for H-10. Owing to the presence of a different sesquiterpene moiety, in dysidenones the H-10 was observed at chemical shift values very far from those reported for dactylospongenones; therefore this stereochemical feature remained unassigned and the stereochemistry arbitrarily drawn in structures **2** and **3** could be interchangeable.

The molecular formula of dysidine (**4**) was established as $C_{23}H_{33}NO_6S$ from HRFABMS data (452.2122, $[M + H]^+$). This formula, along with the chromatographic behavior, suggested the presence of a sulfate group in **4**, which was supported by IR absorption bands (ν_{max} 1250 and 1225 cm^{-1}). NMR data (Experimental Section) clearly indicated the presence of the same rearranged drimane skeleton

Table 2. Effect of Compounds **1**−**4** on Different sPLA$_2$ Activities[a]

	N. naja venom	pancreas		human synovial		bee venom	
compound	% *I* (10 μM)	% *I* (10 μM)	IC$_{50}$ (μM)	% *I* (10 μM)	IC$_{50}$ (μM)	% *I* (10 μM)	IC$_{50}$ (μM)
1	26.0 ± 1.6[c]	86.4 ± 1.9[c]	0.4	100.0 ± 0.0[c]	0.2	99.1 ± 0.6[c]	0.1
2, **3**	11.0 ± 2.0[b]	23.5 ± 2.5[b]		45.7 ± 6.3[c]		1.9 ± 1.7	
4	0.7 ± 0.7	1.2 ± 1.2		73.8 ± 2.4[c]	2.0	33.4 ± 6.3[c]	
manoalide	17.0 ± 1.7[b]	32.3 ± 2.7[c]		93.2 ± 0.2[c]	3.9	62.5 ± 3.8[c]	7.5

[a] Results show percentages of inhibition at 10 μM, and IC$_{50}$ (μM) values determined only for those compounds that reach 50% of inhibition. Mean ± SEM (n = 6). [b] $p < 0.05$. [c] $p < 0.01$.

Table 3. Effect of Compounds **1**−**4** on Human Neutrophil Functions[a]

	elastase degranulation release		chemiluminescence	viability
compound	% *I* (10 μM)	IC$_{50}$ (μM)	% *I* (10 μM)	% *I* (10 μM)
1	68.1 ± 1.4[b]	5.2	40.1 ± 3.1[b]	14.0 ± 2.5[b]
2, **3**	49.7 ± 3.7[b]		47.3 ± 3.2[b]	0.0 ± 0.0
4	90.7 ± 1.6[b]	1.3	44.4 ± 2.0[b]	0.3 ± 0.2

[a] Results show percentages of inhibition at 10 μM, and IC$_{50}$ (μM) values determined only for those compounds that reach 50% of inhibition. Mean ± SEM (n = 6). [b] $p < 0.01$.

found in the other metabolites isolated from this collection of *Dysidea* sp. sponge. ^{13}C NMR data suggested the presence of an amino-*p*-quinone system. Remaining ^{1}H NMR data (δ_H: 3.13, t, J = 7 Hz and 3.62, t, J = 7 Hz) were consistent with the presence of a taurine residue. The proposed structure (**4**) is mainly based on the ^{13}C quinone ring chemical shifts, which parallel those of smenospongidine[7] and other amino-quinone derivatives with a NR group at the C-20 position.

From a pharmacological point of view, PLA$_2$s are the enzymes responsible for arachidonic acid release for the biosynthesis of a great variety of eicosanoids and the platelet-activating factor.[2] It is known that inhibition of PLA$_2$ can modulate the production of different inflammatory mediators. Unfortunately, no potent and absolutely type-specific PLA$_2$ inhibitors are widely available. Compounds **1**, **4**, and a 1:1 mixture of **2** and **3** at 10 μM significantly inhibited human synovial PLA$_2$ (Table 2). Among them, compound **1**, with an IC$_{50}$ value of 0.2 μM, is the most potent but not selective inhibitor toward this enzyme. On the contrary, compound **4**, which possesses a sulfonic group, with an IC$_{50}$ value of 2.0 μM is a selective inhibitor against this enzyme with a higher potency than the reference, nonspecific inhibitor, manoalide. In contrast, compounds **1**−**4** had no inhibitory effects on cPLA$_2$ (data not shown), although this enzyme was partially inhibited by manoalide at 10 μM (44.6 ± 5.6 inhibition, mean ± SEM, n = 6).

On the other hand, compounds **2**−**4** were devoid of significant cytotoxic effects on human neutrophils at concentrations up to 10 μM, as assessed by the mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to formazan (Table 3). In addition compounds **1**, **4**, and a 1:1 mixture of **2** and **3** inhibited some human leukocyte functions such as the degranulation process measured as elastase release and the chemiluminescence response induced by stimulation of neutrophils with 12-*O*-tetradecanoyl phorbol 13-acetate (TPA) (Table 3). Compound **4** elicited a concentration-dependent inhibition of cytochalasin B + *N*-formyl-L-methionil-L-leucyl-L-phenylalanine (fMLP) induced neutrophil degranulation measured as elastase release with an IC$_{50}$ value of 1.3 μM. Compound **4** does behave as a potent and reasonably type-selective human synovial PLA$_2$ inhibitor, and it may be a prototype for the development of more selective agents that can specifically target a single PLA$_2$ enzyme.

Experimental Section

General Experimental Procedures. For general procedures see M. V. D'Auria et al.[8]

Animal Material. The sponge was collected at Lahdu (Santo) in Vanuatu in June 1996 and identified as *Dysidea* sp. (family Dysideidae, order Dictyoceratida) by John Hooper (Queensland Museum, Brisbane, Australia). The voucher specimen was deposited at the Queensland Museum, Brisbane, Australia, under the accession number G306969. It is similar in external morphology to *Dysidea arenaria*, but is bright yellow alive and mauve in ethanol. Growth form is lobate digitate, with a conulose surface composed of uneven tangential cored fibers forming a cobweb-like network between conules. Texture is firm, harsh, and barely compressible in ethanol, with few single oscules on apical and subapical margins of digits, relatively large (>3 mm diameter), each with a raised fleshy membraneous lip. Ectosomal skeleton is membraneous, virtually undifferentiated from hoanosome, with choanosomal fibers pushing the surface up into surface conules. Choanosomal reticulation of fibers is fully charged with sand grains, with mainly larger (primary) fibers dominating over the smaller connecting secondary fibers (uncored). Fiber characteristics are totally obscured by coring sand. Mesohyl collagen is moderately dense and is flocculent (clumped) in nature and contains sparsely dispersed fine sand grains.

Isolation. Lyophilized animals (800 g) were extracted with MeOH to obtain 90 g of a deep purple, amorphous solid, which was subsequently partitioned using a modified Kupchan partition.[4] The $CHCl_3$ (9.8 g) extract was fractionated by Si gel MPLC (Merck Kiesegel 60, 230−400 mesh, 200 g) eluting with 0.2% MeOH/CH_2Cl_2 followed by reversed-phase C$_{18}$ μ-Bondapak HPLC with 0.1% TFA 75% aqueous MeOH to give bolinaquinone (**1**, 2.7 g), dysidotronic acid (7.2 mg), and a 1:1 mixture of dysidenones A and B (**2**, **3**, 6.3 mg). An aliquot (2.0 g) of the BuOH extract (15.6 g) was chromatographed in two runs on a Sephadex LH-20 column (eluent MeOH) to give 22.0 mg of dysidine (**4**) and 18.4 mg of xestoquinol sulfate.[5]

Dysidenones A and B (2, 3): colorless amorphous material; UV (MeOH) λ_{max} (log ϵ) 250 (3.6) nm; IR (KBr) ν_{max} 3500, 1730, 1640 cm^{-1}; ^{1}H and ^{13}C NMR data in Table 1; HRFABMS m/z 391.2462 (calcd for $C_{23}H_{35}O_5$, 391.2484).

Dysidine (4): deep purple crystalline solid; $[\alpha]^{25}_D$ +19 (c 1 , MeOH); UV (EtOH) λ_{max} (log ϵ) 228 (4.43) 334 (4.15) nm; IR (KBr) ν_{max} 3500, 3250, 1565, 1250, 1225 cm^{-1}; ^{1}H (CD_3OD, 500 MHz) δ 5.35 (1H, s, H-19), 5.16 (1H, bs, H-3), 3.62 (2H, t, J = 7.0 Hz, H-22), 3.13 (2H, t, J = 7.0 Hz, H-23), 2.50 (1H, d, J = 12.5 Hz, H-15a), 2.26 (1H, d, J = 12.5 Hz, H-15b), 2.07 (2H, m, H-2), 1.97 (1H, m, H-10), 1,84 (1H, m, H-1a), 1.82 (1H, m, H-7a), 1.56 (3H, s, H-11), 1.48 (1H, m, H-9), 1.46 (1H, m, H-6a), 1.31 (1H, m, H-6b), 1.11 (1H, m, H-7b), 1.04 (3H, d, J = 7 Hz, H-14), 1.00 (3H, s, H-12), 0.93 (3H, d, J = 7.0 Hz, H-13); ^{13}C NMR (CD_3OD, 125 MHz) δ 182.4 (s, C-21), 181.0 (s, C-18),

170.3 (s, C-17), 153.4 (s, C-20), 145.0 (s, C-4), 121.5 (d, C-3), 114.5 (s, C-16), 92.6 (d, C-19), 49.8 (t, C-23), 46.6 (d, C-9), 42.3 (d, C-10), 40.4 (s, C-8), 39.7 (t, C-22), 39.1 (s, C-5), 34.8 (t, C-15), 33.4 (t, C-6), 29.8 (t, C-7), 27.8 (t, C-2), 25.9 (t, C-1), 24.3 (q, C-13), 20.3 (q, C-12), 18.0 (q, C-11), 12.8 (q, C-14); HRFABMS *m/z*, 452.2122 (calcd for $C_{23}H_{34}NO_6S$, 452.2107).

Chemicals and Enzymes. Human synovial recombinant PLA_2 was kindly provided by Zeneca Pharmaceuticals, Macclesfield, Cheshire, UK. Both [9,10-^{3}H]oleic acid and 1-palmitoyl-2-[^{14}C]arachidonyl-*sn*-glycero-3-phosphocholine were purchased from Du Pont (Itisa, Madrid, Spain). The rest of the reagents were obtained from Sigma Chemicals (St. Louis, MO). *E. coli* strain CECT 101 was a gift from Prof. Uruburu, Department of Microbiology, University of Valencia, Spain.

Assay of sPLA$_2$. sPLA$_2$ was assayed by using a modification of the method of Franson et al.[9] *E. coli* strain CECT 101 was seeded in medium containing 1% tryptone, 0.5% NaCl, and 0.6% sodium dihydrogen orthophosphate, pH 5.0, and grown for 6−8 h at 37 °C in the presence of 5 μCi/mL [^{3}H]oleic acid (sp. act. 10 Ci/mmol). After centrifugation at 2500*g* for 10 min, the cells were washed in buffer [0.7 M Tris-HCl, 10 mM $CaCl_2$, 0.1% bovine serum albumin (BSA) pH 8.0], resuspended in saline, and autoclaved for 30−45 min. At least 95% of the radioactivity was incorporated into phospholipids. *Naja naja* venom enzyme, porcine pancreatic, human recombinant synovial enzyme, and bee venom enzyme were diluted in 10 μL of 100 mM Tris-HCl and 1 mM $CaCl_2$ buffer, pH 7.5. Enzymes were preincubated at 37 °C for 5 min with 2.5 μL of test compound solution or its vehicle in a final volume of 250 μL. Incubation proceeded for 15 min in the presence of 10 μL of autoclaved oleate-labeled membranes and was terminated by addition of 100 μL of an ice-cold solution of 0.25% BSA in 100 mM Tris-HCl and 1 mM $CaCl_2$ buffer, pH 7.5, to a final concentration of 0.07% w/v. After centrifugation at 1200*g* for 10 min at 4 °C, the radioactivity in the supernatants was determined by liquid scintillation counting.

Assay of cPLA$_2$. cPLA$_2$ activity was measured as the release of radiolabeled arachidonic acid according to the method of Clark et al.[10] using cytosolic fractions of macrophage-like RAW 264.7 cell line as the source of enzyme and 1-palmitoyl-2-[^{14}C]arachidonyl-*sn*-glycero-3-phosphocholine (57.0 mCi/mmol, 2×10^6 cpm) as substrate. Test compounds were dissolved in methanol and added to the reaction mixture just before the addition of the enzyme solution. The final concentration of methanol in the reaction mixture was less than 1%, which showed no effect on the enzyme activity. The reaction was stopped after a 60 min incubation period at 37 °C by mixing with 0.5 mL of isopropyl alcohol−heptane−0.5 M H_2SO_4 (10:5:1). Hexane (0.7 mL) and water (0.2 mL) were then added, and the solution was vigorously mixed for 15 s. The hexane phase was mixed with 100 mg of Si gel 60 (Merck, 70-230 mesh) and centrifuged, and the radioactivity in each supernatant was measured.

Cytotoxicity Assays. The mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan[11] was used to assess the possible cytotoxic effects of test compounds on human neutrophils. Leukocytes were obtained and purified as previously described.[12]

Chemiluminescence. Neutrophils (2.5×10^6/mL) were mixed with luminol (40 μM) and stimulated with 1 μM 12-*O*-tetradecanoyl phorbol 13-acetate (TPA). The chemiluminescence was recorded in a Microbeta Trilux counter (Wallac, Turku, Finland) after 7 min, previously selected as the time of maximal production.[13]

Elastase Release by Human Neutrophils. Neutrophils (2.5×10^6/mL) were preincubated with test compounds or vehicle for 5 min and then stimulated with cytochalasin B (10 μM) and *N*-formyl-L-methionil-L-leucyl-L-phenylalanine (FMLP, 10 nM) for 10 min at 37 °C. After centrifugation at 1200*g* at 4 °C for 5 min, supernatants were incubated with *N*-*tert*-butoxy-carbonyl-L-alanine *p*-nitrophenyl ester (200 μM) for 20 min at 37 °C.[14] The extent of *p*-nitrophenol release was measured at 414 nm in a microtiter plate reader. Possible direct inhibitory effects on elastase activity were assessed by preincubating test compounds for 5 min with supernatants of cytochalasin B+FMLP-stimulated human neutrophils, followed by addition of substrate and a 20 min incubation at 37 °C.[15]

Statistical Analysis. The results are presented as mean ± SEM. Inhibitory concentration 50% (IC_{50}) values were calculated from at least four significant concentrations ($n = 6$). The level of statistical significance was determined by analysis of variance (ANOVA), followed by Dunnett's *t*-test for multiple comparisons.

Acknowledgment. This contribution is part of the EC project Marine Sciences and Technology MAST III Contract MAS 3-CT95-0032. This work was supported by grants from MURST (PRIN '99) "Chimica dei composti organici di interesse biologico" Rome, Italy, and from CICYT (SAF98-0119), Spanish Ministerio de Educación y Ciencia. Mass spectra were provided by the CRIAS Centro Interdipartimentale di Analisi Strumentale, Faculty of Pharmacy, University of Naples. The staff are acknowledged. The NMR spectra were recorded at CRIAS Centro Interdipartimentale di Analisi Strumentale, Faculty of Pharmacy, University of Naples. We acknowledge the government of Vanuatu for giving us the permission to collect samples there and the Fisheries department for their assistance. We thank the Diving Team from IRD in Nouméa (New Caledonia) for collecting the sample. R.L. was the recipient of a scholarship from Spanish Ministerio de Educación y Cultura.

References and Notes

(1) Giannini, C.; Debitus, C.; Posadas, I.; Payá, M.; D'Auria, M. V. *Tetrahedron Lett.* **2000**, *41*, 3257−3260.
(2) Balsinde, J.; Balboa, M. A.; Insel, P. A.; Dennis, E. A. *Annu. Rev. Pharmacol. Toxicol.* **1999**, *39*, 175−189.
(3) de Guzman, F. S.; Copp, B. R.; Mayne, C. L.; Concepcion, G. P.; Mangalindan, G. C.; Barrows, L. R.; Ireland, C. M. *J. Org. Chem.* **1998**, *63*, 8042−8044.
(4) Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Sigel, C. W. *J. Org. Chem.* **1973**, *38*, 178−179.
(5) Kobayashi, J.; Hirase, T.; Shigemori, H.; Ishibashi, M.; Bae, M.-A.; Tsuji, T.; Sasaki, T. *J. Nat. Prod.* **1992**, *55*, 994−998.
(6) Kushlan, D. M.; Faulkner, D. J.; Parkanyi, L.; Clardy, J. *Tetrahedron* **1989**, *45*, 3307−3312.
(7) Kondracki, M.-L.; Guyot, M. *Tetrahedron* **1989**, *45*, 1995−2004.
(8) D'Auria, M. V.; Giannini, C.; Zampella, A.; Minale, L.; Debitus, C.; Roussakis, C. *J. Org. Chem.* **1998**, *63*, 7382−7388.
(9) Franson, R.; Patriarca, P.; Elsbach, P. *J. Lipid Res.* **1974**, *15*, 380−388.
(10) Clark, J. D.; Milona, N.; Knopf, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 7708−7712.
(11) Gross, S. S.; Levi, R. *J. Biol. Chem.* **1992**, *267*, 25722−25729.
(12) Bustos, G.; Ferrándiz, M. L.; Sanz, M. J.; Payá, M.; Alcaraz, M. J. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1995**, *351*, 298−304.
(13) Terencio, M. C.; Ferrándiz, M. L.; Posadas, I.; Roig, E.; De Rosa, S.; De Giulio, A.; Payá, M.; Alcaraz, M. J. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1998**, *357*, 565−572.
(14) Barrett, A. J. *Methods Enzymol.* **1981**, *80*, 581−588.
(15) Escrig, V.; Ubeda, A.; Ferrándiz, M. L.; Darias, J.; Sánchez, J. M.; Alcaraz, M. J.; Payá, M. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 123−131.

NP000637W