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NEW LEUKOTRIENE B_4 RECEPTOR ANTAGONIST: LEUCETTAMINE A AND RELATED IMIDAZOLE ALKALOIDS FROM THE MARINE SPONGE *LEUCETTA MICRORAPHIS*

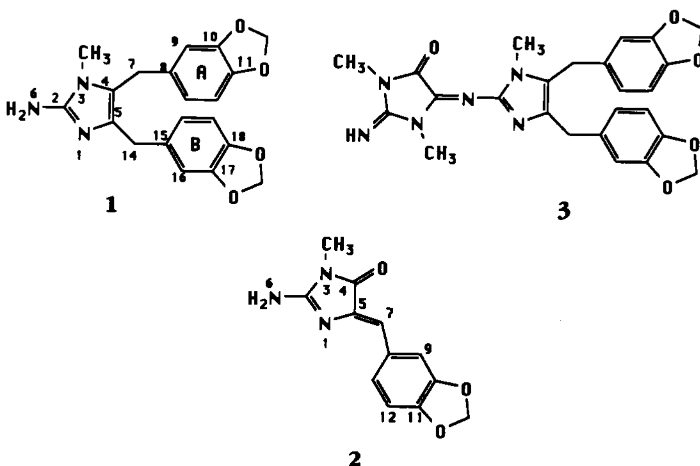
GEORGE W. CHAN,* SEYMOUR MONG, MARK E. HEMLING, ALAN J. FREYER, PRISCILLA H. OFFEN, CHARLES W. DEBROSSE, HENRY M. SARAU, and JOHN W. WESTLEY

Research and Development, SmithKline Beecham Pharmaceuticals, PO Box 1539, King of Prussia, Pennsylvania 19406-0939

ABSTRACT.—Three new imidazole alkaloids, leucettamines A [1] and B [2] and leucettamidine [3], have been isolated from the Palauan sponge *Leucetta microraphis*. Their structures were established on the basis of extensive spectral analyses. Leucettamine A showed potent leukotriene B_4 receptor binding activity ($K_i = 1.3$ μM), while leucettamine B was essentially inactive ($K_i = 100$ μM) and leucettamidine showed significant activity ($K_i = 5.3$ μM). With leucettamine A identified as a pure LTB_4 receptor antagonist, a new structure lead is presented to inflammation therapy.

The important role of leukotrienes as mediators of inflammation and immediate hypersensitivity has long been recognized and delineated (1). Leukotriene B_4 is a non-peptide metabolite of arachidonic acid produced mainly in inflammatory cells (2). It has been implicated in particular as a significant proinflammatory mediator of several common clinical diseases like arthritis (rheumatoid and gouty), cystic fibrosis, psoriasis, and ischemia/perfusion cardiac damage (3–5). Finding ways to antagonize the pathophysiological effect of this proinflammatory mediator has been the focus of many research efforts (6,7). One such logical approach is to block the LTB_4 functional activity with selective receptor antagonists so that the mediator cannot exert its deleterious effect. Receptor antagonists synthesized earlier for this approach were modelled after LTB_4 itself, and most of them lacked selectivity and showed partial agonist activity (8). To look for structurally different and novel LTB_4 receptor antagonists, we have focused our attention on natural product screening. In order to implement high throughput screening, modification of reported procedures (9) was made to enhance our ability to detect molecules with high affinity for the receptor. Additional assays were established to rapidly assess agonist/antagonist activity. In this paper, we report the isolation of a new class of LTB_4 receptor antagonist using this screening effort.

Our LTB_4 receptor binding screen led to the selection of a CH_2Cl_2 extract of an In-



dopacific sponge, *Leucetta microraphis* Haeckel (Calcarea class), for fractionation. The collection of this sponge was made from the Argulpelu Reef in Palau at a depth of 30 meters. Previously, the isolation of a novel pteridine alkaloid leucettidine was reported from a Bermuda collection of this sponge (10). The freshly collected *L. microraphis* was lyophilized and then extracted sequentially with hexane, CH_2Cl_2, and MeOH. The bioactive CH_2Cl_2 extract was subjected to a combination of reversed-phase and silica chromatographies. The bioassay-guided fractionation produced two new imidazole compounds, leucettamine A [**1**] and leucettamidine [**3**], as the active principles of the extract. Also isolated was a related compound leucettamine B [**2**] with no LTB_4 receptor binding activity. Leucettamine A [**1**] was identified by spectral analyses and has since been prepared by total synthesis (J.C. Boehm, J.G. Gleason, I. Pendrak, H.M. Sarau, B.C. Schmidt, J.J. Foley, and W.D. Kingsbury, to be published). Its structure and activity have been confirmed.

RESULTS AND DISCUSSION

Leucettamine A [**1**] was obtained as a yellowish amorphous solid. Its hrfabms spectrum (*m/z* $[M+H]^+$ 366.1455) established a molecular formula of $C_{20}H_{19}N_3O_4$. This formula was consistent with the ^{13}C-nmr GASPE results (Table 1) showing twenty carbon signals, eleven of which were associated with a total of seventeen protons. These data suggested two exchangeable protons in the molecule, which were subsequently confirmed by a deuterium exchange experiment using ND_3 in cims. The ^{1}H 2D decoupling data suggested two methylenedioxybenzyl groups, both with ABX substitution patterns. The aromatic protons at δ 6.65 (1H, d, $J=0.7$ Hz), 6.66 (1H, dd, $J=7.6$, 0.7 Hz) and 6.76 (1H, d, $J=7.6$ Hz) formed an ABX system A. The resonances at δ 6.81 (1H, dd, $J=7.9$, 1.7 Hz), 6.86 (1H, d, $J=1.7$ Hz), and 6.72 (1H, d, $J=7.9$ Hz) formed ABX system B. The two benzylic methylenes at δ 4.00 and 3.84 are associated with the ABX systems, as was first evidenced by their nuclear Overhauser interactions with the protons at δ 6.65, 6.66 and δ 6.81, 6.86, respectively. Small but significant nOe effects also established the association of the dioxygenated methylene at δ 5.96 with ABX system A, and the methylene at δ 5.93 with system B. In support of the presence of the methylenedioxybenzyl functionality, ion fragments at *m/z* 244, 230, and 135 (Figure 1) were observed in the eims. With the accounting of two methylenedioxybenzyl groups, a $C_4H_5N_3$ fragment remained to be elucidated for leucettamine A. The presence of an imidazole moiety for this $C_4H_5N_3$ fragment became apparent with the consideration of an *N*-methyl group (δ 3.28, s) and two exchangeable protons. A 2-amino-4,5-disubstituted imidazole was subsequently established by ^{1}H-^{13}C correlation experiments. As indicated by COLOC, three-bond proton-carbon couplings were observed (Table 1) for δ 3.28 to C-2 and C-4, δ 6.76 to C-8

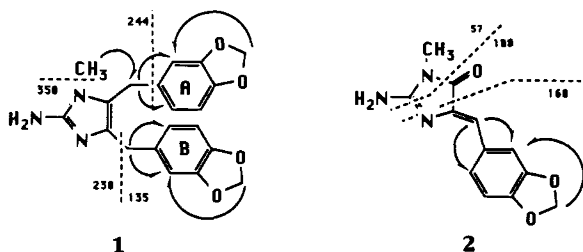


FIGURE 1. NOe data and mass spectral fragmentation patterns of leucettamine A [**1**] and leucettamine B [**2**].

TABLE 1. ^{1}H-nmr and ^{13}C-nmr Data for Leucettamine A [1].[a]

Position	δ ^{13}C[b]	δ ^{1}H (integ., mult., J in Hz)[b]	^{1}H long-range coupling to ^{13}C[c]
2	148.4		
4	122.2		
5	123.8		
7	28.4	4.00 (2H, brs)	C-4, C-5, C-8, C-9
8	131.9		
9	109.3	6.65 (1H, d, 0.7)	
10	149.0		
11	147.4		
12	109.0	6.76 (1H, d, 7.6)	C-8, C-10
13	121.9	6.66 (1H, dd, 0.7, 7.6)	C-9, C-11
14	30.12	3.84 (2H, brs)	C-4, C-5, C-15, C-16
15	132.9		
16	109.8	6.86 (1H, d, 1.7)	
17	148.8		
18	147.3		
19	108.9	6.72 (1H, d, 7.9)	C-15, C-17
20	122.5	6.81 (1H, dd, 1.7, 7.9)	C-16, C-18
3-NMe	29.7	3.28 (3H, s)	C-2, C-4
6-NH_2		7.84 (brs)	
10-OCH_2O-	102.0	5.96 (2H, s)	
17-OCH_2O-	101.8	5.93 (2H, s)	

[a]Recorded in Me_2CO-d_6. All chemical shifts were reported with respect to TMS (δ 0).
[b]Assignments were based on GASPE, HETCOR, COLOC, ^{1}H homodecoupling, and nOe difference experiments.
[c]Assignments were based on COLOC data.

and C-10, and δ 6.66 to C-9 and C-11. With the strong two-bond couplings observed for δ 4.00 with C-4 and C-8 and δ 3.84 with C-5 and C-15, the two benzyl attachments are unambiguously assigned. Due to the presence of the amino group at C-2, two tautomers are therefore possible for leucettamine A [**1**]. With the establishment of its structure, leucettamine A was noted to be closely related to the literature compound naamine A (12).

The compound leucettamine B [**2**] was obtained as a creamy colored solid. Its hrfabms spectrum established a molecular formula of $C_{12}H_{11}N_3O_3$ (m/z $[M+H]^+$ 246.0879). Taking two exchangeable protons into account, this formula is in good agreement with the number of carbons and protons as indicated in the nmr spectra (Table 2). ^{1}H-nmr data showed an ABX system with signals at δ 6.82 (d, J = 8.1 Hz), 7.33 (dd, J = 8.1, 1.6 Hz), and 8.04 (d, J = 1.6 Hz). Also observed in the proton spectrum were three singlets, typical of N-Me, methylenedioxy, and olefinic protons, at δ 3.13 (3H), 6.01 (2H), and 6.42 (1H), respectively. From the ^{13}C GASPE spectrum, nine carbons in the formula of $C_{12}H_{11}N_3O_3$ were accounted for by the benzene ring, methylenedioxy, olefin, and N-methyl signals. The remaining three carbons in the formula could be accounted for by an imidazolone ring with three sites of unsaturation. With an imidazole structure assigned for compound **1** isolated from the same sponge, the mass spectral fragmentation pattern (Figure 1) readily supported an imidazolone structure for **2**. The amide functionality was supported by a carbon signal at δ 170.5 and a strong ir band at 1696 cm^{-1}. The mass spectral fragments at m/z 188, 160, and 57 clearly precluded the possibility of the C=C at the C-4 position and the C=O at the C-5 position. The substitution pattern on the imidazolone was confirmed by HETCOR and COLOC experiments. As indicated in Table 2, the N-methyl protons showed a

TABLE 2. ^{1}H-nmr and ^{13}C-nmr Data of Leucettamine B [2].[a]

Position	δ ^{13}C[b]	δ ^{1}H (integ., mult., J in Hz)[b]	^{1}H long-range coupling to ^{13}C[c]
2	160.2		
4	170.5		
5	140.4		
7	114.6	6.42 (1H, s)	C-4, C-9, C-13
8	131.7		
9	110.8	8.04 (1H, d, 1.6)	C-13
10	148.6		
11	148.1		
12	108.8	6.82 (1H, d, 8.1)	C-8, C-10
13	126.3	7.33 (1H, dd, 1.6, 8.1)	C-9, C-11
3-NMe	25.7	3.13 (3H, s)	C-2, C-4
10-OCH_2O	102.0	6.01 (2H, s)	
6-NH_2		6.72 (2H, brs)	

[a]Recorded in Me_2CO-d_6. All chemical shifts were reported with respect to TMS (δ 0).
[b]Assignments were bsed on GASPE, HETCOR, COLOC, ^{1}H homodecoupling, and nOe difference experiments.
[c]Assignments were based on COLOC data.

three-bond correlation to the carbons at δ 170.5 and δ 160.2; the olefinic proton at δ 6.42 also gave a three-bond correlation to the carbon resonance at δ 170.5 in addition to the aromatic signals at δ 110.8 and 126.3. All the quaternary carbons in the methylenedioxyphenyl moiety were assigned in Table 2 according to the COLOC results. The stereochemistry at the exocyclic double bond was determined on the basis of ^{13}C-^{1}H coupling constants. Exocyclic double bonds for model compounds in the literature (12) showed $J_{C\text{-}H}$ 12–15 Hz for the *E* form and 5–8 Hz for the *Z* form. By selective decoupling of the *N*-methyl protons, the coupling at C-4 arose from the olefinic proton at δ 6.42 and not the *N*-methyl. This coupling was a doublet (J = 5.3 Hz) and indicated therefore a *Z* stereochemistry.

Leucettamidine [3] was isolated only in small quantities. Its hrfabms data (m/z $[M+H]^+$ 489.1869) indicated a formula of $C_{25}H_{24}N_6O_5$, differing from leucettamine A by an element of $C_5H_5N_3O$. Except for this element, the rest of the leucettamidine molecule was virtually identical to leucettamine A [1] as indicated by spectral comparisons of their ^{1}H 2D COSY and nOe data. Leucettamidine showed two additional *N*-Me groups at δ 3.13 and 3.16. These methyl signals did not give nOe enhancements to the other signals in the molecule, suggesting that they resided in a remote ring system. Attempts to obtain ^{13}C-nmr data for leucettamidine failed as a result of the instability of the natural product. With the precedents of imino-imidazolone moieties reported for related compounds in the literature (11), such a function was tentatively assigned for leucettamidine [3], thus accounting for the $C_5H_5N_3O$ element.

Sponges are known to be rich in nitrogenous metabolites (13). Several imidazole alkaloids had been isolated from other sponge species. The naamines and naamidines from the Red Sea sponge *Leucetta chagosensis* were reported to have antifungal activity (11,14). The alkaloid clathridine from the Napoli sponge *Clathrina clathrus* was found to show also antimycotic activity (15). From Saipan and Guam *Leucetta* sp., pyronaamidine was isolated and shown to possess KB cell cytotoxicity (16).

A membrane receptor binding assay (17) with [^{3}H]-LTB_4 was initially used to guide our fractionation and to evaluate our isolated natural products for comparative potency. Leucettamine A [1] gave an IC_{50} of 4.0 μM (K_i = 1.3 μM), and leucettamidine [3] gave an IC_{50} of 15.6 μM (K_i = 5.3 μM). Leucettamine B [2] was found to

be essentially inactive ($K_i = 100$ μM) in the assay. Among the three imidazole alkaloids, the most potent leucettamine A [1] was further evaluated in a human whole cell receptor binding assay (19) and was found to have high affinity for the receptors on the U937 cells ($IC_{50} = 0.75$ μM). Furthermore, when evaluated for agonist/antagonist activity in fura-2 loaded U937 cells using procedures as described in the literature (18, 19), leucettamine A [1] showed no agonist activity. It did not induce a Ca^{+2} transient on its own but was able to block the LTB_4-induced Ca^{+2} transient with an IC_{50} of 4.6 μM. This result suggests that leucettamine A [1] is a pure antagonist of the LTB_4 receptor.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were obtained on a Beckman DU-7 spectrometer. Ir spectra were taken on a Nicolet 20DXB Fourier transform ir spectrometer equipped with a DTGS detector. ^{1}H-nmr and ^{13}C-nmr spectra were obtained with a Bruker AMX400 instrument operating at 400.13 and 100.62 MHz, respectively. The fabms were measured on a VG ZAB-1F-HF mass spectrometer equipped with a standard fab ion source. The samples were applied in a matrix of dithiothreitol and dithioerythritol. The desorption eims were obtained on a Finnigan MAT 4610 mass analyser with the sample applied to a direct probe heated at 10 mA/sec with a source temperature of 150° and ionization energy of 70 eV.

COLLECTION, EXTRACTION, AND ISOLATION.—The sponge sample was collected from Argulpelu Reef, Palau at a depth of 30 m in 1981. This Indopacific sponge was identified as *L. microraphis* by Dr. Rob van Soest at the Institute of Taxonomic Zoology, University of Amsterdam. Voucher specimens (ZMA register number POR.8786) are located at the Zoology Museum of Amsterdam. The freeze-dried sponge (1.1 kg) was cut into small pieces before sequential extraction with 4 liters each of hexane, CH_2Cl_2, and MeOH. The active CH_2Cl_2 extract (5.1 g) was chromatographed on a Whatman Partisil ODS column eluting with a step gradient of MeCN in 0.2% TFA. The inactive compound **2** was eluted in the 15% MeCN fractions, and the active compounds **1** and **3** were eluted in the 30–40% MeCN fractions. Compound **2** was further purified by chromatography with MeOH-CH_2Cl_2 (6:94) on silica (EM Science, Si gel 60, 70–230 mesh) and by crystallization in MeOH. The yield of leucettamine B [**2**] was 11 mg as a cream-colored solid. The separation of compounds **1** and **3** was accomplished using reversed-phase hplc (Rainin Dynamax ODS) in which compound **1** was eluted by 30% MeCN/0.2% TFA and compound **3** by 35% MeCN/0.2% TFA. The final purification of leucettamine A [**1**] was by means of chromatographies on silica [Rainin Dynamax Silica, MeOH-CH_2Cl_2 (10:90)] and reversed-phase (Beckman Ultrasphere ODS, 36% MeCN/0.2% TFA). The purified **1** was a pale yellowish solid in a yield of 15.8 mg. Compound **3** after final purification by silica [Rainin Dynamax silica, MeOH-CH_2Cl_2 (10:90)] was obtained as a yellowish solid in a yield of 2 mg.

Leucettamine A [**1**].—Ir ν max (KBr) 3600–3100 br, 1681, 1503, 1490, 1444, 1246, 1203, 1186, 1040 cm^{-1}; uv λ max (MeOH) 205 nm (ε 4281), 285 (796); hrfabms *m/z* ($[M+H]^+$ 366.1455 ($C_{20}H_{20}N_3O_4$ requires 366.1454); eims *m/z* (rel. int.) $[M]^+$ 365 (100), 350 (18), 244 (22), 242 (48), 230 (82), 135 (58).

Leucettamine B [**2**].—Ir ν max (KBr) 3600–3100 br, 1696, 1679, 1661, 1607, 1586, 1569, 1484, 1263, 1155, 1037 cm^{-1}; uv λ max (MeOH) 205 nm (ε 2316), 237 (1831), 363 (3712); hrfabms *m/z* $[M+H]^+$ 246.0879 ($C_{12}H_{12}N_3O_3$ requires 246.0879); eims *m/z* (rel. int.) $[M]^+$ 245 (62), 188 (3), 160 (20), 107 (6), 85 (4), 57 (100).

Leucettamidine [**3**].—Hrfabms *m/z* $[M+H]^+$ 489.1869 ($C_{25}H_{25}N_6O_5$ requires 489.1886); ^{1}H nmr (400 MHz, MeCO-d_6) δ 3.13 (s, 3H), 3.16 (s, 3H), 3.56 (s, 3H), 4.04 (s, 2H), 4.15 (s, 2H), 5.96 (s, 2H), 6.71 (d, 1H, $J=1.1$ Hz), 6.72 (dd, 1H, $J=1.1$, 8.6 Hz), 6.77 (d, 1H, $J=8.0$ Hz), 6.79 (d, 1H, $J=8.6$ Hz), 6.80 (dd, 1H, $J=1.1$, 8.0 Hz), 6.87 (d, 1H, $J=1.1$ Hz).

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