New Imidazole Alkaloids from the Indonesian Sponge *Leucetta chagosensis*

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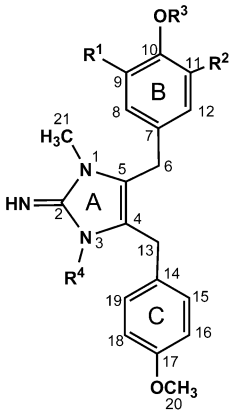
Chemical investigation of the sponge *Leucetta chagosensis* collected in Indonesia afforded five new imidazole alkaloids, naamine F (**2**), naamine G (**3**), kealiinine A (**6**), kealiinine B (**7**), and kealiinine C (**8**), in addition to the known compound naamine A (**1**). Naamine G (**3**) exhibited strong antifungal activity against the phytopathogenic fungus *Cladosporium herbarum* and also showed mild cytotoxicity against mouse lymphoma (L5178Y) and human cervix carcinoma (HeLa) cell lines. In the brine shrimp assay, kealiinine A (**6**) was more active than naamine G (**3**).The structures of the new compounds were unambiguously established by 1D and 2D NMR and MS data.

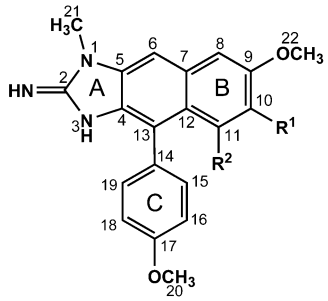
Among the calcareous sponges, the genus *Leucetta* has up to now received the widest attention.[1] Chemical investigation of sponges of the genus *Leucetta* has led to the isolation of interesting imidazole alkaloids such as naamines,[2−6] isonaamines,[2,3] naamidines,[3,7,10−12] and isonaamidines.[8,13] The alkaloids of these groups are similar in that each analogue possesses a central imidazole ring to which one or two substituted benzyl moieties are attached at C-4 and C-5, or a methyl group at the N-3 position. In addition, biosynthetically related compounds, which include dorimidazole,[14] leucettamine,[3,8] preclathridines,[13,14] and kealiiquinone,[5,10] have also been isolated. Recently, a Fijian collection yielded unique chiral 2-aminoimidazole and spirocyclopentimidazolidines known as calcaridine and spirocalcaridine, respectively.[9] Imidazole alkaloids were shown to exhibit interesting biological activities such as antimicrobial,[2−4,12] anticryptococcal,[6] inhibition of nitric oxide synthase,[10] and cytotoxic activity.[4] It was revealed that naamidine A exhibited antitumor activity by regulation of ERK1 and ERK2, resulting in stimulation of p21 level and consequently arresting cells at the G1 phase, which is a new mechanism for anticancer agents.[15]

Results and Discussion

The present study is focused on the sponge *Leucetta chagosensis* collected from South Sulawesi, Indonesia. The dried methanolic extract of the freeze-dried sponge tissue was subjected to solvent partitioning between hexane, EtOAc, BuOH, and H_2O phases. Further chromatographic isolation was carried out with the dried BuOH and EtOAc fractions, which were found to be biologically active in the brine shrimp assay. The BuOH fraction was subjected to reversed-phase silica gel column chromatography, which yielded naamine A (**1**) and the new naamine congeners F (**2**) and G (**3**). The EtOAc fraction was chromatographed over a Sephadex column and afforded a new group of naamine congeners that are also structurally related to kealiiquinone, for which we propose the names kealiinines



1 R^1 = H, R^2 = H, R^3= H, R^4 = H
2 R^1 = OCH_3, R^2 = H, R^3= H, R^4 = H
3 R^1 = OCH_3, R^2 = OCH_3, R^3= H, R^4 = H
4 R^1 = OH, R^2 = H, R^3= CH_3, R^4 = CH_3
5 R^1 = OH, R^2 = OH, R^3= CH_3, R^4 = H



6 R^1= OH, R^2= H
7 R^1= OCH_3, R^2= H
8 R^1= OCH_3, R^2= OCH_3

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Table 1. NMR Data of Naamines F (**2**) and G (**3**) in DMSO-d_6, 500 MHz

	2			**3**		
no.	δ_H (m, *J* in Hz)	δ_C [a]	HMBC	δ_H (m, *J* in Hz)	δ_C (m)	HMBC
2	7.35 (br s, N*H*)	145.9		7.55 (s, N*H*)	146.2 s	
3				12.47 (br s, N*H*)		C-2, C-4
4		122.7			122.1 s	
5		122.4			121.7 s	
6	3.85 (s)		C-4, C-8, C-12	3.86 (s)	27.9 t	C-4, C-8, C-12
7					127.0 s	
8	6.60 (d, 2.0 Hz)	110.3		6.31 (s)	105.5 d	C-7, C-9, C-10, C-12
9		147.5			148.1 s	
10				8.23 (br s, O*H*)	134.2 s	
11	6.67 (d, 8.2 Hz)				148.1 s	
12	6.50 (dd, 8.2, 2.0 Hz)	127.8	C-10	6.31 (s)	105.5 d	C-7, C-8, C-10, C-11
13	3.80 (s)		C-15, C-19	3.81 (s)	27.6 t	C-5, C-15, C-19
14					130.3 s	
15	7.10 (d, 8.2 Hz)	129.0	C-16	7.17 (d, 8.5 Hz)	129.3 d	C-17, C-19
16	6.80 (d, 8.2 Hz)	116.0		6.85 (d, 8.5 Hz)	113.9 d	C-15, C-17, C-18
17		157.8			157.9 s	
18	6.80 (d, 8.2 Hz)	116.0		6.85 (d, 8.5 Hz)	113.9 d	C-16, C-17, C-19
19	7.10 (d, 8.2 Hz)	129.0	C-18	7.17 (d, 8.5 Hz)	129.3 d	C-15, C-17
20	3.72 (s, OCH_3)		C-17	3.70 (s, OCH_3)	55.1 q	C-17
21	3.12 (s, NCH_3)		C-2, C-5	3.16 (s, NCH_3)	29.4 q	C-2, C-5
22	3.63 (s, OCH_3)		C-9	3.60 (s, OCH_3)	55.8 q	C-9
23				3.60 (s, OCH_3)	55.8 q	C-11

[a] Carbon assignments were determined indirectly from the HMBC, which did not allow a complete assignment.

A (**6**), B (**7**), and C (**8**). Compound **1** was obtained as a dark brown amorphous powder. It was identified as naamine A on the basis of its UV spectrum, HRESIMS, ^{1}H NMR, and HMBC spectral data.[2,3]

Naamine F (**2**) was isolated together with compound **1** as a yellowish brown amorphous substance. The ESIMS spectrum of compound **2** showed a pseudo-molecular ion peak at *m/z* 354 $[M + H]^+$, which was compatible with the molecular formula $C_{20}H_{24}N_3O_3$ as established through (+)-HRESIMS. The inspection of the ^{1}H NMR spectrum of compound **2** (Table 1) showed that it was closely related to that of naamine A (**1**).[2] It showed two signals at δ_H 7.10 (2H, d, 8.2 Hz) and 6.80 (2H, d, 8.2 Hz), which were assigned to an AA′BB′ spin system of ring C and a singlet signal at δ_H 3.12 for an *N*-methyl function. Two methylene singlet signals at δ_H 3.85 and 3.80 were observed, which were assigned to CH_2-6 and CH_2-13, respectively. The low-field chemical shifts of these suggested that they belonged to benzylic groups. An exchangeable proton for a N*H* group at δ_H 7.35 was also observed. ^{1}H NMR showed an additional methyl singlet signal at δ_H 3.72, which was assigned to a methoxyl group. The presence of a *para*-methoxybenzyl moiety as in naamine A was established from the HMBC correlations of the methylene singlet at δ_H 3.80 (CH_2-13) with the carbon signal at δ 129.0 for C-15 and C-19, while the methoxyl singlet at δ_H 3.72 (OCH_3-20) correlated with C-17 at δ_C 157.8. Irradiation of the methoxyl singlet (OCH_3-20) at δ_H 3.72 gave a NOE response for the AA′BB′ methine protons (H-16/18) at δ_H 6.80, while the methylene proton at δ_H 3.80 enhanced the AA′BB′ methine protons (H-15/H-19) at δ_H 7.10, which confirmed the substitution pattern in ring C in compound **2**. The ESIMS/MS spectrum gave a fragment peak at *m/z* 246 $[M - C_7H_7O]^+$ due to the loss of a methoxyphenyl moiety, which further confirmed the presence of this substructure. The presence of a 2-imino-4,5-dibenzylimidazole was evident from the chemical shifts of C-2, C-4, and C-5 at δ_C 145.9 (s), 122.7 (s), and 122.4 (s), respectively.[5] The HMBC correlations of NCH_3-21 with C-2 and C-5 proved its attachment at N-1, which was further established through the NOE of the NCH_3 singlet at δ_H 3.12 on the proton (H-8) at δ_H 6.60. However, due to the broadness of the signal at δ_H 6.50, which was assigned for H-12, an NOE effect of NCH_3-21 on this proton was not discernible. The major difference between compounds **1** and **2** was that the second AA′BB′ spin system found in naamine A (**1**) for ring B was replaced by an ABC spin system which was comparable to that of naamine B (**4**).[3] The signals at δ_H 6.60 (1H, d, 2.0 Hz), 6.50 (1H, dd, 8.2 and 2.0 Hz), and 6.67 (1H, d, 8.2 Hz) signified the presence of a 1,3,4-trisubstituted benzene. An additional methoxyl singlet was also observed at δ_H 3.63, which satisfied the 30 mass unit difference in the molecular weight when compared with naamine A. Irradiation of the methoxyl singlet at δ_H 3.63 gave a NOE effect at δ_H 6.60, which identified the position of the methoxyl group, OCH_3-22, at C-9. Compound **2** is at present the sixth member of the group known as naamines, and it was thus named naamine F.

Naamine G (**3**) was isolated as a yellowish brown oil, whose molecular formula was determined to be $C_{21}H_{25}N_3O_4$ from the HREIMS. The ESIMS/MS spectrum showed fragmentation peaks at *m/z* 262 $[M - C_8H_9O]^+$ and *m/z* 216 $[M - C_9H_{11}O_3]^+$ due to the subsequent loss of a *para*-methoxybenzyl function and the *para*-hydroxy-3,5-dimethoxybenzyl unit, respectively. ^{13}C NMR and DEPT spectra of compound **3** (Table 1) showed 21 carbons, which consisted of nine quaternary carbons, six methines, two methylenes, and four methyls. The ^{1}H NMR spectrum (Table 1) was very simple and showed signals for 25 protons, which comprised the three exchangeable protons at δ_H 12.47, 8.23, and 7.55, a pair of AA′BB′ doublets at δ_H 7.17 and 6.85, an aromatic singlet for two protons at δ_H 6.31, two methylene singlets at δ_H 3.86 and 3.81, methoxyl singlets at δ_H 3.70 (3H) and 3.60 (6H), and a *N*-methyl singlet at δ_H 3.16. Comparison of the NMR data of compound **3** with those of naamines A (**1**) and F (**2**) showed that the compounds were again closely related. Inspection of their ^{1}H and ^{13}C NMR spectra showed that the AA′BB′ spin system for ring C and the 1-*N*-methyl imidazole ring were present in all congeners, and this was confirmed through a series of NOE experiments that were performed on all the isolated naamine derivatives (Figures 1 and 2). Compound **3**, however, contained a symmetrically 1,3,4,5-substituted benzyl moiety, which is comparable to that of naamine E (**5**).[4] The symmetry of ring B was evidenced from the ^{1}H NMR singlet signals observed at δ_H 3.60, which integrated

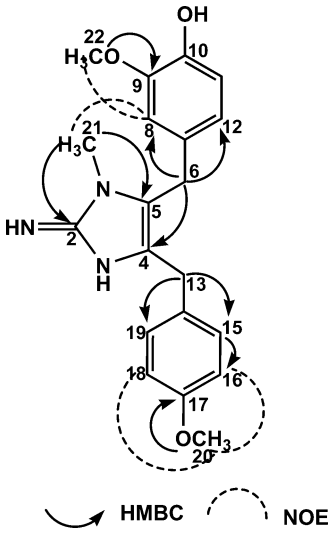

Figure 1. Important HMBC and NOE correlations of naamine F (**2**).

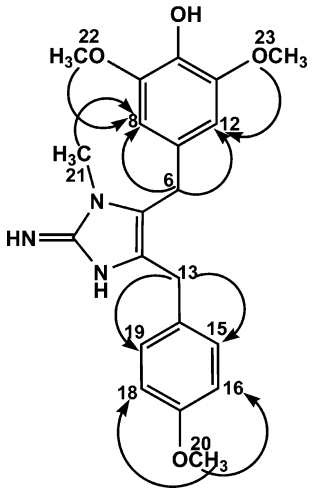

Figure 2. Important NOE correlations of naamine G (**3**).

for two OCH_3 functions, and two aromatic methine protons resonating at δ_H 6.31, which gave both an HMQC and HMBC cross-peak with the methine carbon at δ_C 105.5. The ^{1}H NMR data for ring B were also compatible with the ^{13}C NMR spectral data, which consisted of a quaternary signal at δ_C 148.1 for C-9 and C-11, a methine signal at δ_C 105.5 for C-8 and C-12, and a methyl signal at δ_C 55.8 for OCH_3-22 and OCH_3-23. Through the HMBC spectrum, the attachment of the methoxyl groups was established to be at C-9 and C-11. This was shown by the HMBC correlations of the methine singlet at δ_H 6.31 (H-8 and H-12) with the methylene carbon at δ_C 27.9 (C-6) and also with quaternary carbons at δ_C 127.0 (C-7), 134.2 (C-10), and 148.1 (C-9 and C-11). The carbon resonance at δ_C 148.1 further gave a correlation with the methoxyl singlets at δ_H 3.60 assigned to OCH_3-22 and OCH_3-23. The NOE experiment finally confirmed the substitution pattern of ring B. Irradiation of the methyl singlet at δ_H 3.60 for OCH_3-22 and OCH_3-23 gave an enhancement of the methine singlet at δ_H 6.30 for H-8 and H-12. Compound **3**, named naamine G, has a disposition of the oxygen substituents similar to that of naamine E (**5**) but differed in the proportions of hydroxyl and methoxyl functions.

Kealiinine A (**6**) was isolated as a yellowish brown powder. The ESIMS showed a pseudo-molecular ion peak at *m/z* 350 $[M + H]^+$ corresponding to the molecular formula $C_{20}H_{20}N_3O_3$ as determined by (+)HRESIMS. The ^{1}H and ^{13}C NMR data of **6** (Table 2) were comparable to those of the naamines and those of 2-aminokealiiquinone.[5] Inspection of the ^{1}H and ^{13}C NMR spectral data of compound **6** showed that the AA′BB′ spin system for ring C and the 1-*N*-methyl imidazole ring were also present. Although shifted to low field, the ^{1}H NMR signals at δ_H 7.35 (2H, d, 8.5 Hz) and 7.18 (2H, d, 8.5 Hz) also indicated the presence of an AA′BB′ spin system. The presence of the 1-*N*-methyl imidazole ring was confirmed from the ^{13}C NMR quaternary signals at δ_C 152.0, 125.0, and 129.5 and the NCH_3 resonance at δ_C 28.1. The attachment of the methyl function at N-1 was established from the HMBC correlation of the NCH_3 singlet signal at δ_H 3.67 with the quaternary carbon signals at δ_C 152.0 (C-2) and 129.5 (C-5) in addition to its ROESY correlation with that of the methine singlet at δ_H 7.71 for H-6. The ^{13}C NMR spectrum of **6** showed the presence of 20 carbons, which consisted of

Table 2. NMR Data of Kealiinines A (**6**), B (**7**), and C (**8**) in DMSO-d_6 and MeOD, 500 MHz

	6 (in DMSO-d_6)			**7** (in MeOD)	**8** (in MeOD)
no.	δ_H	δ_C	HMBC (δ_H to δ_C)	δ_H	δ_H
2	8.30 (s, N*H*)	152.0 s			
3	12.10 (br s, N*H*)				
4		125.0 s			
5		129.5 s			
6	7.71 (s)	104.4 d	C-4, C-8, C-12	7.90 (s)	7.81 (s)
7		125.0 s			
8	7.40 (s)	106.5 d	C-6, C-7, C-9, C-10, C-12	7.47 (s)	7.33 (s)
9		148.1 s			
10	9.49 (s, O*H*)	146.5 s			
11	6.95 (s)	107.0 d	C-7, C-9, C-10, C-12, C-13	7.10 (s)	
12		124.8 s			
13		118.5 s			
14		127.0 s			
15	7.35 (d, 8.5 Hz)	131.5 d	C-13, C-17, C-19	7.47 (d, 8.5 Hz)	7.35 (d, 8.5 Hz)
16	7.18 (d, 8.5 Hz)	114.2 d	C-14, C-17, C-18	7.24 (d, 8.5 Hz)	7.12 (d, 8.5 Hz)
17		159.0 s			
18	7.18 (d, 8.5 Hz)	114.2 d	C-14, C-16, C-17	7.24 (d, 8.5 Hz)	7.12 (d, 8.5 Hz)
19	7.35 (d, 8.5 Hz)	131.5 d	C-13, C-15, C-17	7.47 (d, 8.5 Hz)	7.35 (d, 8.5 Hz)
20	3.88 (s, OCH_3)	55.5 q	C-17	3.96 (s, OCH_3)	3.94 (s, OCH_3)
21	3.67 (s, NCH_3)	28.1 q	C-2, C-5,	3.79 (s, NCH_3)	3.78 (s, NCH_3)
22	3.90 (s, OCH_3)	55.8 q	C-9	4.02 (s, OCH_3)	4.03 (s, OCH_3)
23				3.77 (s, OCH_3)	3.89 (s, OCH_3)
24					3.30 (s, OCH_3)

10 quaternary carbons, seven methine carbons, and three methyl carbons as determined from its DEPT spectrum. The ^{13}C NMR data of compound **6** revealed a structural similarity to 2-aminokealiiquinone except for the replacement of the two carbonyl groups at δ_C 181.8 and 182.3 ppm by two methine carbons at δ_C 106.5 (C-8) and 107.0 (C-11), which was compatible with the methine singlets at δ_H 7.40 (H-8) and 6.95 (H-11) in its ^1H NMR spectrum, as shown by their direct correlations in the HMQC spectrum. The hydrogenation occurring at C-11 caused an upfield shift for C-13 to 118.5 ppm compared to 129.5 ppm in 2-aminokealiiquinone. The loss of the keto functions was confirmed from the HMBC spectrum (Table 2), which showed correlations of δ_H 7.40 (H-8) with C-6, C-7, C-9, C-10, and C-12 (δ_C 104.4, 125.0, 148.1, 146.5, and 124.8, respectively) and δ_H 6.95 (H-11) with C-7, C-9, C-10, C-12, and C-13 (δ_C 125.0, 148.1, 146.5, 124.8, and 118.5, respectively). This was further established from the ROESY correlations of δ_H 7.40 (H-8) with the singlet at δ_H 7.71 (H-6) and of δ_H 6.95 (H-11) with the AA′BB′ doublet at δ_H 7.35 (H-15/19) in ring C. Apart from the additional aromatic methine singlets, the ^1H NMR data of **6** were comparable to those of 2-aminokealiiquinone. The absence of a third methoxyl singlet suggested that the OCH_3 attached at C-10 in 2-aminokealiiquinone had been replaced by a hydroxyl function that afforded a broad singlet signal at δ_H 9.49 in kealiinine A (**6**). In kealiinine A, the methyl signals at δ_H 3.67, 3.88, and 3.90 corresponded to the presence of a NCH_3 and two OCH_3 functions, respectively. The attachment of the methoxyl groups was determined from the HMBC spectrum through the correlations of δ_H 3.90 (OCH_3-22) with δ_C 148.1 for C-9 of ring B and of δ_H 3.88 (OCH_3-20) with δ_C 159.0 for C-17 of ring C. This was further confirmed by the ROESY correlations of the methoxyl singlets at δ_H 3.90 and 3.88 with the methine singlet δ_H 7.40 for H-8 and with the AA′BB′ doublet protons at δ_H 7.18 for H-16/18, respectively. The major difference from the spectral data of the naamines was the absence of the methylene signals in the ^1H and ^{13}C NMR spectra. Compound **6** appeared to be derived from the naamines, particularly naamine F, by ring closure between the methylene carbon at position 13 and C-12 of ring B followed by aromatization of C-6 and C-13. The HMBC correlations of δ_H 7.35 (H-15/19) with δ_C 118.5 (C-13) and 159.0 (C-17) confirmed the attachment of ring C at C-13. Irradiation of δ_H 7.35 (H-15/19) showed an enhancement of δ_H 6.95 (H-11), confirming ring C is attached at C-13. Compound **6** is the first member of a new class of compounds related to kealiiquinone and was thus named kealiinine A.

Kealiinine B (**7**) was isolated as a dark brown amorphous solid. The ESIMS spectrum showed a pseudo-molecular ion peak at *m*/*z* 364 $[M + H]^+$ corresponding to the molecular formula $C_{21}H_{22}N_3O_3$ established by (+)HRESIMS. Its UV spectrum and ^1H NMR, COSY, and NOE data (Table 2 and Figure 3) showed that it is closely related to kealiinine A (**6**). Due to the very small yield, the structure could be established only from a series of 1D ^1H NOE performed on all the protons observed in the ^1H NMR spectrum (Figure 3). This spectrum showed three aromatic methine singlet signals at δ_H 7.90, 7.47, and 7.10 for H-6, H-8, and H-11, respectively, and four methyl singlets at δ_H 3.79, 3.96, 4.02, and 3.77, which were assigned to NCH_3-21, OCH_3-20, OCH_3-22, and OCH_3-23, respectively. Detailed comparison of the ^1H NMR spectrum (Table 2) of compound **7** with that of kealiinine A revealed that the two compounds were quite similar, except for the presence of an additional methoxyl group. This suggested the possible methylation of the hydroxyl function at C-10, which was also compatible with the 14 mass unit difference in molecular weight between kealiinine A (**6**) and the latter compound. This was confirmed, as irradiation of the singlet at δ_H 3.77 gave an enhancement of the methine singlet at δ_H 7.10 (H-11), which confirmed its attachment to C-10. The similarity between compound **7** and kealiinine A was also revealed by the almost identical results of the NOE experiments performed on both compounds (Figure 3). Irradiation of the methyl protons at δ_H 3.79 (NCH_3-21), 3.96 (OCH_3-20), and 4.02 (OCH_3-22) enhanced the methine singlets for H-6 (δ_H 7.90), H-16/18 (δ_H 7.24), and H-8 (δ_H 7.47), while irradiation of these methine singlets gave the same NOE effects on the complimentary methyl resonances. The NOE effect of H-15/19 on H-11 also confirmed that ring C is bound at C-13. From these data, it was concluded that compound **7** is the 10-methoxylated congener of kealiinine A and was named kealiinine B.

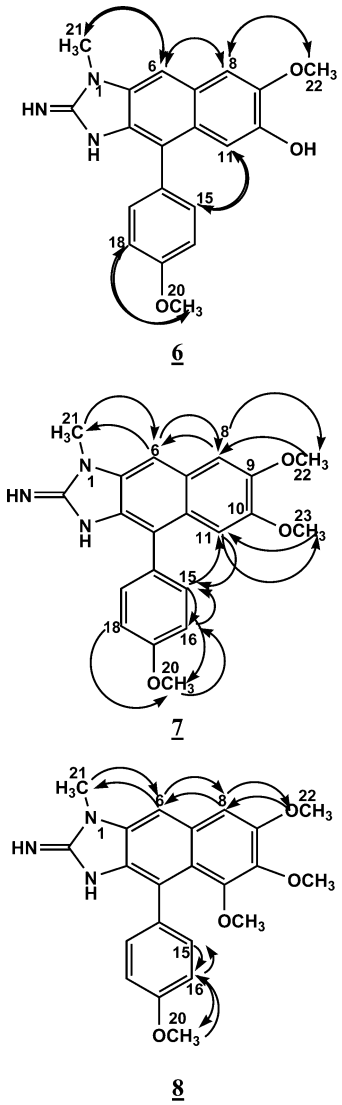

Figure 3. Important NOE correlations observed for the methoxy functions in kealiinines (A) **6**, (B) **7**, and (C) **8**.

Kealiinine C (**8**) was isolated as a dark brown amorphous solid. The ESIMS spectrum showed a molecular ion peak at *m*/*z* 394 $[M + H]^+$, which was 30 mass units higher than that of kealiinine B (**7**) and was compatible with the molecular formula $C_{22}H_{24}N_3O_4$ established by (+)-HRESIMS. Its UV spectrum and ^1H NMR data (Table 2) showed that it is closely related to kealiinines A (**6**) and B

(**7**) except for the disappearance of the methine singlet at ca. 7.00 ppm previously assigned to H-11 in the latter compounds and also the emergence of an additional NCH_3 or OCH_3 methyl signal. The 30 atomic mass unit difference could be accounted for by the presence of an additional methoxyl group at C-11. A NOE experiment showed an enhancement effect of δ_H 3.78 (NCH_3-1) on H-6 (δ_H 7.81) and vice versa, which confirmed the presence of a methyl substituent at N-1. Irradiation of the methine singlet at δ_H 7.81 enhanced the singlet at δ_H 7.33 assigned to H-8, which when irradiated, also enhanced the methyl signal at δ_H 4.03 assigned to OCH_3-22, confirming the methoxyl substituent at C-9. A NOE effect on the AA′BB′ doublet protons at δ_H 7.12 for H-16/18 was observed when the methoxyl singlet at δ_H 3.94 was irradiated and confirmed the methoxyl bound to C-17. This also established that ring C in compound **8** is identical to that in kealiinines A and B. Although no NOE effect was observed when the methoxyl signals at δ_H 3.89 and 3.30 were irradiated, the above data were compatible only with further methoxyl groups at C-10 and C-11.

Numerous marine imidazole alkaloids have recently been isolated, and many exhibit some form of antimicrobial and/or antitumor activity.[15] On the basis of the bioassays conducted in this study, the new compound naamine G (**3**) exhibited mild cytotoxicity toward mouse lymphoma (L5178Y) and human cervix carcinoma (HeLa) cell lines, while it was found to be inactive toward the rat brain tumor PC12 cell line. At a concentration of 10 μg/mL, naamine G showed antiproliferation activities of 46% and 29% for L5178Y and HeLa cell lines (controls set at 100%), respectively. In the brine shrimp assay, kealiinine A (**6**) was more active than naamine G (**3**). At concentrations of 20 μg/mL, kealiinine A gave a mortality rate of 50%, while naamine G resulted in a mortality of only 10%. This implied that kealiinine A was responsible for the observed activity of the ethyl acetate extract in the brine shrimp lethality test. Naamine G (**3**) was also found to be strongly active against the fungal strain *C. herbarum*, exhibiting a zone of inhibition of 20 mm in the agar plate diffusion assay (20 μg/disk), while kealiinine A was inactive by comparison.

The chemistry of *Leucetta* sponges is dominated by the imidazole alkaloids. However, the occurrence of two chemotypes has been observed among Indo-Pacific *Leucetta* sponges.[8] The sponges would accumulate either 2-aminoimidazoles or amino polyene analogues.[17−19] So far, no report has documented the occurrence of both types of analogues in the same sample collection.[8] Chemical investigation of *L. chagosensis* from different geographical locations such as the Red Sea,[2,3] Micronesia,[5] the Mariana Islands,[7] the Great Barrier Reef,[4] and the Fiji Islands[4] afforded only 2-aminoimidazole alkaloids. In the present study, *L. chagosensis* from Indonesia also yielded 2-aminoimidazole congeners, which include new naamine derivatives and the hitherto unreported class of analogues, the kealiinines. The structures of the kealiinines are additional evidence of the biogenetic relationship of kealiiquinone to the naamines, which was previously postulated.[10] The kealiinines are probably biogenetic intermediates of the naamines leading to the kealiiquinones.

Experimental Section

General Experimental Procedures. UV spectra were measured in methanol on a Perkin-Elmer UV/vis lambda spectrophotometer. ^{1}H (1D, 2D COSY) and ^{13}C (1D, 2D HMBC) NMR spectra were recorded on Bruker AM 300, ARX 400, or DRX 500 NMR spectrometers. Mass spectra were recorded on a Finningan MAT TSQ-7000 mass spectrometer, while HREIMS were obtained on a Finningan MAT 900 mass spectrometer. Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements. TLC was performed on plates precoated with silica gel F_{254} (Merck, Darmstadt, Germany). For semipreparative HPLC, a HPLC system (Merck, Darmstadt, Germany) coupled with UV detector L7400 (UV detection at 280 nm) was used. The separation column (8 × 250 mm) was prepacked with Eurosphere 100 C_{18} (Knauer, Berlin, Germany). The compounds were eluted with mixtures of MeOH and H_2O at a flow rate of 5 mL/min.

Animal Material. The sponge *Leucetta chagosensis* (Dendy) belongs to the class Calcarea, order Leucettida, family Leucittidae. It is a lemon-yellow, soft sponge collected near the coast of Kapoposang Island, Indonesia, on August 1997 at a depth of 41 ft. A voucher specimen has been deposited in the Zoological Museum, Amsterdam, under the registration number ZMA POR 17167.

Isolation. The freeze-dried sponge (111 g of dry weight) was extracted several times with MeOH and then with CH_2Cl_2. The total extract (4 g) was evaporated to dryness and partitioned between aqueous MeOH and the following organic solvents: hexane, EtOAc, and BuOH. The BuOH fraction (870 mg) was subjected to reversed-phase silica gel column chromatography (MeOH/H_2O/TFA, 50:50:0.1%) to yield naamine A (**1**, 1.1 mg), naamine F (**2**, 1.1 mg), and naamine G (**3**, 7.5 mg). The EtOAc fraction (692 mg) was chromatographed over a Sephadex LH20 column using MeOH as eluent to give kealiinine A (**6**, 3.5 mg), kealiinine B (**7**, 1.2 mg), and kealiinine C (**8**, 1.2 mg). Further purification of the compounds was accomplished by semipreparative HPLC.

Naamine A (1): dark brown amorphous solid; UV λ_{max} (MeOH) 230, 278 nm; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.35 (1H, br s, N*H*), 7.13 (2H, d, J = 8.2 Hz, H-15/19), 6.98 (2H, d, J = 8.2 Hz, H-8/12), 6.83 (2H, d, J = 8.2 Hz, H-16/18), 6.66 (2H, d, J = 8.2 Hz, H-9/11), 3.88 (2H, s, CH_2-6), 3.73 (2H, s, CH_2-13), 3.68 (3H, s, OCH_3), 3.12 (3H, s, NCH_3); (+)ESIMS m/z 324 [M + H]$^+$; HRESIMS m/z 324.1720 [M + H]$^+$ (calcd for $C_{19}H_{22}N_3O_2$, 324.1712).

Naamine F (2): dark brown amorphous solid; UV λ_{max} (MeOH) 226, 279 nm; ^{1}H NMR data, see Table 1; (+)ESIMS m/z 354 [M + H]$^+$; HRESIMS m/z 354.1817 [M + H]$^+$ (calcd for $C_{20}H_{24}N_3O_3$, 354.1818).

Naamine G (3): yellowish brown oil; UV λ_{max} (MeOH) 230, 276 nm; ^{1}H NMR and ^{13}C NMR data, see Table 1; (+)ESIMS m/z 384 [M + H]$^+$; EIMS m/z 383 [M]$^+$ (100), 353 (20), 331 (3), 262 (7), 230 (12), 216 (35), 186 (7), 167 (7), 143 (11), 121 (14), 84 (19), 69 (68), 44 (94); HREIMS m/z 383.1848 [M]$^+$ (calcd for $C_{21}H_{25}N_3O_4$, 383.1845).

Kealiinine A (6): dark brown amorphous solid; UV λ_{max} (MeOH) 250, 314, 338 nm; ^{1}H NMR and ^{13}C NMR data, see Table 2; (+)ESIMS m/z 350 [M + H]$^+$; HRESIMS m/z 350.1507 [M + H]$^+$ (calcd for $C_{20}H_{20}N_3O_3$, 350.1505).

Kealiinine B (7): dark brown amorphous solid; UV λ_{max} (MeOH) 250, 314, 338 nm; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.64 (1H, s, H-6), 7.32 (1H, s, H-8), 7.22 (2H, d, J = 8.5 Hz, H-15/19), 7.10 (1H, s, H-11), 6.97 (2H, d, J = 8.5 Hz, H-16/18), 3.91 (3H, s, OCH_3-22), 3.89 (3H, s, OCH_3-20), 3.74 (3H, s, NCH_3-21), 3.72 (3H, s, OCH_3-23); (+)ESIMS m/z 364 [M + H]$^+$; HRESIMS m/z 364.1650 [M + H]$^+$ (calcd for $C_{21}H_{22}N_3O_3$, 364.1661).

Kealiinine C (8): dark brown amorphous solid; UV λ_{max} (MeOH) 250, 314, 338 nm; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.66 (1H, s, H-6), 7.33 (2H, d, J = 8.5 Hz, H-15/19), 7.19 (1H, s, H-8), 7.09 (2H, d, J = 8.5 Hz, H-16/18), 3.99 (3H, s, OCH_3-22), 3.85 (3H, s, OCH_3-20), 3.83 (3H, s, OCH_3-23), 3.73 (3H, s, NCH_3-21), 3.40 (3H, s, OCH_3-24); (+)ESIMS m/z 394 [M + H]$^+$; HRESIMS m/z 394.1765 [M + H]$^+$ (calcd for $C_{22}H_{24}N_3O_4$, 394.1767).

Bioassays. Antimicrobial Assay. Sterile filter paper disks were impregnated with 20 μg of the samples using methanol as the carrier solvent. The impregnated disks were then placed on agar plates previously inoculated with *Bacillus subtilis* (DSM 2109), *Escherichia coli* (DSM 10290), *Cladosporium herbarum* (DSM 63422), and *Cladosporium cucumerinum*

(DSM 62122). Solvent controls were run against each organism. After the plates were incubated at 37 °C for 24 h, antimicrobial activity was recorded as clear zones (in mm) of inhibition surrounding the disk. The test sample was considered active when the zone of inhibition was greater than 7 mm.

Cytotoxicity Assay. Antiproliferative activity was examined against several cell lines and was determined through an MTT assay as described earlier.[20,21] Activity against brine shrimp, *Artemia salina*, was determined as previously outlined.[22]

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