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Tetrahedron
Tetrahedron 63 (2007) 2432–2438

Njaoamines A–F, new cytotoxic polycyclic alkaloids from the haplosclerid sponge *Reniera* sp.

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Received 14 December 2006; revised 8 January 2007; accepted 8 January 2007
Available online 12 January 2007

Abstract—Six new cytotoxic polycyclic alkaloids containing an 8-hydroxyquinoline moiety, njaoamines A–F (**1**–**6**), have been isolated from the 2-propanol extract of the sponge *Reniera* sp. collected in Tanzania. Their structures were determined by extensive analysis of their spectroscopic features, particularly 1D and 2D NMR spectra recorded in Py-d_5. Cytotoxicity of the compounds isolated was evaluated against a panel of three human tumor cell lines.

1. Introduction

Sponges have proven to be a rich source of compounds with interesting biological and structural properties. In particular, specimens belonging to the order Haplosclerida constitute a rich source of alkylpyridine and alkylpiperidine derived alkaloids.[1] These compounds possess interesting biological properties and have also been considered as chemotaxonomic markers of this order.[2] This group of metabolites includes, among others, a class of pentacyclic cytotoxic alkaloids belonging to the 'ingenamine' family represented by ingenamines A–G[3] and ingamines A and B,[4] isolated from *Xestospongia ingens* and *Pachychalina* sp., as well as xestocyclamines A and B,[5] isolated from *Xestospongia* sp., and keramaphidin B, obtained from *Amphimedon* sp.[6] The structures of these compounds include a tricyclic nitrogenated core and two carbon bridges of different lengths. Baldwin and Whitehead anticipated the existence of such compounds in their elegant proposal for the biogenetic origin of the manzamines from a bis-3-alkyldihydropyridine macrocyclic precursor.[7]

In the course of our ongoing program for the search of new antitumor agents from marine sources, the 2-propanol extracts of the sponge *Reniera* sp. were found to display cytotoxicity against the human tumor cell lines A-549, HT-29, and MDA-MB-231. Bioassay-guided fractionation of these extracts led to the isolation of njaoamines A–F (**1**–**6**), a group of cytotoxic alkaloids containing a tricyclic nitrogenated core similar to that of the ingenamines as well as two carbon bridges, one of them embedding an 8-hydroxyquinoline moiety, which differentiates this family from other alkaloids previously isolated from different sponges of the order Haplosclerida. Examples of other metabolites containing a quinoline moiety that have been isolated from sponges include halitulin isolated from *Halichlona tulearensis*,[8] containing two 7,8-dihydroxyquinoline systems, and the *Aplysina aerophoba* metabolite 3,4-dihydroxyquinoline-2-carboxylic acid.[9] However, to the best of our knowledge, the njaoamines are the first compounds containing an 8-hydroxyquinoline moiety that has been isolated from sponges. Herein we report the isolation, structural characterization, and cytotoxic properties of this new family of marine metabolites.

2. Results and discussion

The crude 2-propanol extract of *Reniera* sp. was resuspended in H_2O–MeOH 4:1 and subjected to a solvent–solvent partition with *n*-hexane, EtOAc, and *n*-BuOH. Reversed phase C_{18} chromatography of the *n*-BuOH extract and repeated preparative and semipreparative reversed phase HPLC of selected active fractions from this chromatography led to the isolation of compounds **1**–**6** as their TFA salts.

The choice of the NMR solvent was of particular importance in the structural determination of this family of compounds. ^{1}H and ^{13}C NMR experiments run using MeOH-d_4 or DMSO-d_6 resulted in poorly resolved spectra, with broad signals unsuitable for structural studies. Eventually, it was found that spectra with well-resolved resonances could be obtained using Py-d_5 as the NMR solvent (Tables 1–3).

Keywords: Sponge metabolites; Polycyclic alkaloids; Quinoline; Cytotoxicity.
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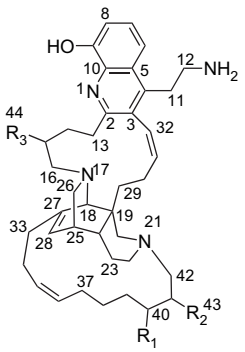
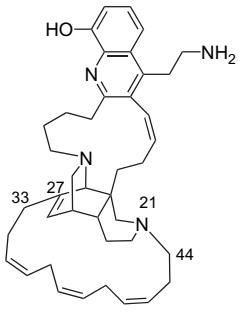
doi:10.1016/j.tet.2007.01.013



1 R_1=OH, R_2=Me, R_3=H
2 R_1=OH, R_2=R_3=Me
3 R_1= R_2= R_3=H
4 R_1=R_3=H, R_2=Me



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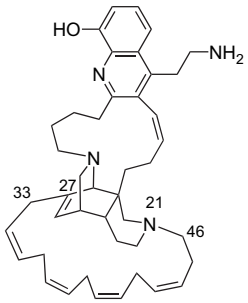

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The most polar compound of the extract, njaoamine A (**1**), was isolated as an optically active pale yellow gum. A pseudomolecular ion in the (+)-HRMALDIMS at *m/z* 625.4479 and the presence of 40 signals in the ^{13}C NMR

Table 1. ^{1}H NMR data (Py-d_5, 500 MHz) for njaoamines A–D (**1**–**4**)

No	^{1}H (multiplicity, *J*)			
	1	**2**	**3**	**4**
N1				
2				
3				
4				
5				
6	7.66 (d, 8.0)	7.66 (dd, 8.0, 1.0)	7.60 (br d, 8.3)	7.63 (d, 8.0)
7	7.28 (dd, 8.0, 7.5)	7.28 (t, 8.0)	7.30 (dd, 8.3, 7.7)	7.28 (dd, 8.5, 7.5)
8	7.26 (d, 7.5)	7.25 (dd, 8.0, 1.5)	7.28 (br d, 7.7)	7.25 (d, 7.5)
9				
10				
11	3.83 (m), 3.72 (m)	3.85 (ddd, 12.0, 12.0, 5.5), 3.74 (m)	3.82 (ddd, 11.6, 11.6, 5.0), 3.61 (m)	3.80 (m), 3.67 (m)
12	3.61 (m)	3.64 (m)	3.54 (m), 3.46 (m)	3.57 (m)
13	3.19 (m), 2.96 (ddd, 13.0, 12.9, 4.3)	3.16 (ddd, 13.5, 13.5, 5.0), 3.09 (m)	3.14 (m), 2.98 (ddd, 13.0, 12.8, 4.8)	3.17 (m), 2.95 (m)
14	2.35 (m), 1.50 (m)	2.56 (m), 1.16 (m)	2.35 (m), 1.53 (m)	2.30 (m), 1.52 (m)
15	1.29 (m)	1.41 (m)	1.30 (m)	1.29 (m)
16	2.40 (m), 1.95 (m)	2.41 (dd, 13.0, 7.5), 1.68 (dd, 13.0, 12.0)	2.42 (br d, 12.8), 1.98 (m)	2.39 (m), 1.96 (m)
N17				
18	2.60 (br s)	2.68 (br s)	2.66 (br s)	2.62 (br s)
19				
20	3.42 (d, 12.7), 1.88 (m)	3.44 (d, 12.0), 1.91 (br d, 13.5)	3.46 (m), 1.96 (m)	3.36 (d, 12.0), 1.80 (m)
N21				
22	3.68 (m), 3.02 (m)	3.68 (m), 3.04 (br d, 14.0)	3.59 (m), 3.05 (m)	3.66 (m), 2.97 (m)
23	1.50 (m), 1.15 (m)	1.52 (m), 1.18 (m)	1.60 (m), 1.12 (m)	1.56 (m), 1.13 (m)
24	1.17 (m)	1.19 (m)	1.16 (m)	1.17 (m)
25	2.09 (m)	2.12 (br d, 6.5)	2.10 (m)	2.09 (m)
26	3.04 (m), 1.76 (m)	3.09 (br d, 9.5), 1.80 (br d, 9.5)	3.06 (m), 1.78 (m)	3.03 (br d, 9.0), 1.76 (m)
27				
28	5.85 (d, 5.7)	5.88 (d, 6.5)	5.82 (d, 6.0)	5.84 (d, 6.0)
29	2.25 (m), 1.94 (m)	2.28 (m), 1.96 (m)	2.24 (t, 12.5), 1.90 (m)	2.21 (t, 13.5), 1.92 (m)
30	2.84 (m), 1.91 (m)	2.90 (m), 1.96 (m)	2.76 (m), 1.93 (m)	2.81 (m), 1.92 (m)
31	6.05 (dd, 10.9, 10.3)	6.05 (t, 9.5)	5.98 (dd, 11.0, 9.6)	6.02 (br t, 10.0)
32	6.43 (d, 10.9)	6.40 (dd, 11.3, 2.0)	6.43 (dd, 11.0, 2.0)	6.42 (d, 10.5)
33	1.91 (m), 1.26 (m)	2.00 (m), 1.32 (m)	1.90 (m), 1.22 (m)	1.91 (m), 1.23 (m)
34	2.23 (m), 1.92 (m)	2.24 (m), 1.97 (m)	2.04 (m), 1.92 (m)	2.11 (m), 1.92 (m)
35	5.41 (m)	5.44 (m)	5.34 (m)	5.36 (m)
36	5.39 (m)	5.41 (m)	5.34 (m)	5.33 (m)
37	2.11 (m), 1.78 (m)	2.12 (m), 1.82 (m)	1.95 (m), 1.55 (m)	2.00 (m), 1.62 (m)
38	1.50 (m), 0.89 (m)	1.53 (m), 0.91 (m)	0.94 (m), 0.76 (m)	1.00 (m), 0.82 (m)
39	1.51 (m), 1.43 (m)	1.52 (m), 1.44 (m)	1.50 (m), 1.20 (m)	1.04 (m)
40	3.65 (m)	3.64 (m)	1.10 (m)	1.04 (m)
41	1.98 (m)	2.00 (m)	1.80 (m), 1.60 (m)	1.78 (m)
42	3.31 (d, 13.0), 3.22 (m)	3.32 (d, 13.0), 3.23 (dd, 13.0, 9.5)	3.13 (m), 2.88 (m)	2.84 (m)
43	1.53 (d, 5.9)	1.54 (d, 6.5)		1.17 (br d, 5.0)
44		1.12 (d, 6.5)		

Table 2. ^{13}C NMR data (Py-d_5, 125 MHz) for njaoamines A–F (**1–6**)

No	δ_C Multiplicity					
	1	**2**	**3**	**4**	**5**	**6**
N1						
2	158.6 C	158.0 C	158.1 C	158.0 C	158.0 C	158.0 C
3	132.0 C	131.7 C	131.5 C	131.5 C	131.5 C	131.4 C
4	142.6 C	142.2 C	142.2 C	142.1 C	142.1 C	142.0 C
5	127.5 C	127.5 C	127.0 C	127.0 C	126.9 C	126.9 C
6	115.0 CH	114.6 CH	114.6 CH	114.5 CH	114.6 CH	114.6 CH
7	128.0 CH	127.1 CH	127.6 CH	127.5 CH	127.6 CH	127.7 CH
8	110.9 CH	110.3 CH	110.5 CH	110.4 CH	110.4 CH	110.5 CH
9	154.4 C	153.8 C	154.0 C	153.9 C	154.0 C	154.1 C
10	138.1 C	137.5 C	137.6 C	137.5 C	137.6 C	137.6 C
11	28.8 CH_2	28.4 CH_2	28.4 CH_2	28.3 CH_2	28.4 CH_2	28.2 CH_2
12	39.7 CH_2	39.3 CH_2	39.3 CH_2	39.1 CH_2	39.3 CH_2	39.2 CH_2
13	38.7 CH_2	35.3 CH_2	38.3 CH_2	38.2 CH_2	38.3 CH_2	38.3 CH_2
14	26.1 CH_2	32.5 CH_2	25.8 CH_2	25.6 CH_2	25.9 CH_2	25.5 CH_2
15	27.3 CH_2	29.4 CH	26.8 CH_2	26.8 CH_2	26.8 CH_2	26.5 CH_2
16	56.7 CH_2	65.3 CH_2	56.3 CH_2	56.3 CH_2	56.2 CH_2	55.8 CH_2
N17						
18	57.4 CH	57.5 CH	56.8 CH	56.8 CH	56.4 CH	55.0 CH
19	44.2 C	43.6 C	43.8 C	43.7 C	43.5 C	43.6 C
20	51.0 CH_2	50.6 CH_2	49.7 CH_2	50.3 CH_2	49.7 CH_2	48.3 CH_2
N21						
22	51.9 CH_2	51.5 CH_2	49.5 CH_2	51.0 CH_2	49.5 CH_2	50.0 CH_2
23	23.9 CH_2	23.3 CH_2	24.0 CH_2	23.3 CH_2	24.3 CH_2	24.1 CH_2
24	42.4 CH	42.2 CH	41.5 CH	41.8 CH	41.5 CH	41.5 CH
25	37.1 CH	36.6 CH	36.8 CH	36.6 CH	36.9 CH	37.0 CH
26	57.4 CH_2	57.3 CH_2	56.8 CH_2	56.9 CH_2	56.8 CH_2	56.3 CH_2
27	142.6 C	143.7 C	143.8 C	143.6 C	142.7 C	140.9 C
28	122.2 CH	121.6 CH	122.5 CH	121.9 CH	123.0 CH	124.3 CH
29	37.2 CH_2	37.0 CH_2	36.6 CH_2	36.7 CH_2	36.4 CH_2	35.4 CH_2
30	24.2 CH_2	23.6 CH_2	23.5 CH_2	23.5 CH_2	23.6 CH_2	23.6 CH_2
31	137.2 CH	136.6 CH	136.7 CH	136.6 CH	136.7 CH	136.3 CH
32	124.8 CH	124.5 CH	124.5 CH	124.3 CH	124.6 CH	124.4 CH
33	37.7 CH_2	37.5 CH_2	36.9 CH_2	37.0 CH_2	36.2 CH_2	34.0 CH_2
34	24.0 CH_2	23.8 CH_2	24.4 CH_2	24.0 CH_2	24.7 CH_2	125.9 CH
35	131.0 CH	130.5 CH	129.8 CH	129.9 CH	128.6 CH	129.6 CH
36	129.6 CH	129.2 CH	129.7 CH	129.4 CH	128.8 CH	25.7 CH_2
37	28.0 CH_2	27.5 CH_2	26.7 CH_2	27.0 CH_2	25.7 CH_2	127.6 CH
38	27.2 CH_2	26.7 CH_2	27.8 CH_2	28.6 CH_2	128.2 CH	127.6 CH
39	36.0 CH_2	35.6 CH_2	23.8 CH_2	25.8 CH_2	127.6 CH	26.1 CH_2
40	74.9 CH	74.3 CH	25.4 CH_2	34.1 CH_2	26.2 CH_2	128.0 CH
41	32.9 CH	32.5 CH	19.7 CH_2	26.4 CH	131.9 CH	127.7 CH
42	62.9 CH_2	64.5 CH_2	57.5 CH_2	64.8 CH	123.1 CH	26.1 CH_2
43	18.3 CH_3	17.7 CH_3		18.3 CH_3	22.2 CH_2	131.6 CH
44		17.3 CH_3			57.5 CH_2	123.9 CH
45						22.6 CH_2
46						57.4 CH_2

spectrum were consistent with a molecular formula of $C_{40}H_{56}N_4O_2$ and 15 degrees of unsaturation. The ^{13}C NMR spectrum (Table 2) contained 15 low field signals attributable to olefinic and/or C=N carbons, accounting for eight degrees of unsaturation. Compound **1** must therefore be heptacyclic. The rest of the signals present in the ^{13}C NMR were assigned to an oxygenated methine (δ_C 74.9 ppm) and 24 aliphatic and/or nitrogenated carbons (4 methines, 18 methylenes, 1 quaternary carbon and 1 methyl group according to the HSQC spectrum).

The presence of a 2,3,4-trisubstituted 8-hydroxyquinoline moiety in the molecule was inferred from the presence of signals for three adjacent aromatic protons in the ^1H NMR spectrum (Table 1) at δ_H 7.66 (d), 7.28 (dd), and 7.26 (d) ppm (H-6, H-7, and H-8, respectively) and HMBC correlations observed for these protons and those of the substituents at C-2, C-3, and C-4 (Fig. 1). The substituent at C-4 was identified to be a 2-aminoethyl chain on the basis of ^1H–^1H couplings observed between H-11 and H-12, the carbon chemical shifts for C-11 and C-12, and the HMBC correlations shown in Figure 1.

A second substructure identified in the molecule through analysis of the COSY, HMBC, and selected 1D TOCSY spectra (Fig. 2) was a tricyclic core containing two nitrogen atoms, similar to that described in the ingenamine family of alkaloids.[3–5] NOESY correlations confirmed the same relative stereochemistry as in the ingenamines for this central core. This substructure was connected to the 8-hydroxyquinoline moiety through correlations observed in the COSY, 1D TOCSY, and HMBC spectra. Indeed, 1D TOCSY correlations for the olefinic protons H-31 and H-32 and COSY correlations established the partial sequence from C-29 to C-32. This latter carbon was connected to C-3 of the quinoline moiety through HMBC correlations observed between H-32 and C-3 and C-4, and between H-31 and C-3 (Fig. 1). On the other hand, C-29 was linked to the quaternary carbon C-19 of the tricyclic core on the basis of HMBC cross-peaks observed between H-29 and C-19,

Table 3. ^{1}H NMR data (Py-d_5, 500 MHz) for njaoamines E (**5**) and F (**6**)

No	^{1}H (multiplicity, *J*)	
	5	**6**
N1		
2		
3		
4		
5		
6	7.60 (br d, 8.3)	7.79 (br d, 8.3)
7	7.35 (dd, 8.3, 7.7)	7.43 (dd, 8.3, 7.7)
8	7.28 (dd, 7.7, 0.8)	7.34 (br d, 7.7)
9		
10		
11	3.79 (ddd, 12.1, 11.9, 5.2), 3.55 (m)	3.81 (ddd, 12.0, 12.0, 5.0), 3.65 (ddd, 12.0, 12.0, 5.0)
12	3.51 (m), 3.44 (m)	3.57 (ddd, 12.0, 12.0, 5.0), 3.51 (ddd, 12.0, 12.0, 5.0)
13	3.15 (ddd, 12.8, 4.0, 4.0), 2.98 (ddd, 13.0, 13.0, 4.9)	3.13 (ddd, 12.7, 4.1, 3.9), 2.89 (ddd, 13.0, 12.7, 4.7)
14	2.30 (m), 1.48 (m)	2.30 (m), 1.46 (m)
15	1.30 (m)	1.23 (m)
16	2.42 (m), 1.93 (m)	2.30 (m), 1.91 (m)
N17		
18	2.76 (br s)	2.67 (br s)
19		
20	3.42 (d, 12.2), 2.19 (d, 12.2)	3.23 (d, 12.0), 2.26 (d, 12.0)
N21		
22	3.60 (m), 3.05 (m)	3.62 (m), 3.00 (m)
23	1.56 (m), 1.17 (m)	1.53 (m), 1.22 (m)
24	1.12 (m)	1.11 (m)
25	2.10 (m)	2.08 (m)
26	3.02 (d, 9.0), 1.72 (dd, 9.0, 1.9)	2.99 (br d, 9.0), 1.65 (dd, 9.0, 2.0)
27		
28	5.88 (br d, 5.9)	5.88 (br d, 6.2)
29	2.26 (dd, 13.7, 11.8), 1.94 (m)	2.28 (dd, 14.4, 12.0), 1.99 (m)
30	2.74 (m), 1.87 (m)	2.67 (m), 1.79 (ddd, 12.6, 12.0, 10.8)
31	6.02 (ddd, 11.1, 10.6, 1.4)	6.03 (ddd, 10.9, 10.8, 1.5)
32	6.40 (dd, 11.1, 2.0)	6.42 (dd, 10.9, 1.4)
33	1.86 (m), 1.52 (m)	2.60 (dd, 16.7, 7.6), 2.41 (dd, 16.7, 6.8)
34	2.08 (m), 1.92 (m)	5.28 (ddd, 10.5, 7.6, 6.8)
35	5.31 (m)	5.39 (m)
36	5.19 (ddd, 10.4, 7.0, 6.6)	2.52 (m), 2.30 (m)
37	2.47 (m), 2.38 (m)	5.06 (dt, 11.0, 7.0)
38	5.33 (m)	5.15 (dt, 11.0, 7.0)
39	5.33 (m)	2.55 (m)
40	2.70 (m), 2.46 (m)	5.36 (m)
41	5.53 (ddd, 10.1, 7.9, 7.3)	5.36 (m)
42	5.24 (m)	2.70 (m)
43	2.61 (m), 2.52 (m)	5.56 (dt, 10.6, 7.3)
44	3.24 (ddd, 12.7. 12.7, 4.3), 2.98 (ddd, 12.7, 10.9, 5.2)	5.32 (m)
45	—	2.64 (m), 2.50 (m)
46	—	3.32 (ddd, 11.8, 11.4, 4.8), 3.05 (m)

C-20 and C-24, and between H-18, H-20 and H-24 and C-29 (Fig. 2). The stereochemistry of the $\Delta^{31,32}$ double bond was determined to be *Z* on the basis of a coupling constant of 10.9 Hz observed between both protons and a correlation present in the NOESY spectrum. C-2 was linked to N-17 through a four carbon linear chain. COSY correlations and long-range couplings in the HMBC between H-13 and C-14 and C-15, and between one of the H-16 protons and C-14 established the partial sequence from C-13 to C-16. Both H-13 protons displayed HMBC correlations with C-2 and C-3, which attached C-13 to carbon C-2 of the quinoline moiety (Fig. 1). The carbon chemical shift of C-16 (56.7 ppm) and HMBC cross-peaks between one of the

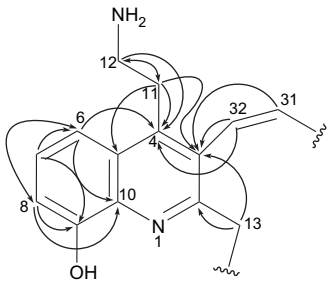

Figure 1. HMBC (H to C) correlations for the 2,3,4-trisubstituted 8-hydroxyquinoline moiety of compound **1**.

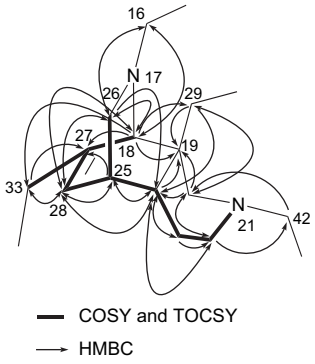

Figure 2. COSY, 1D TOCSY, and HMBC correlations observed in the tricyclic core of njaoamine A.

H-16 protons and C-26 and C-18 and between H-18 and one of the H-26 protons and C-16 determined the linkage of C-16 to the nitrogen atom N-17.

The remaining signals present in both ^{1}H and ^{13}C NMR spectra accounted for the presence in the molecule of six methylenes (one of them nitrogenated), an oxygenated methine, a double bond, a doublet methyl group, and an aliphatic methine. A detailed inspection of the correlations observed in the HMBC, 1D TOCSY, and COSY spectra suggested the arrangement of these groups as depicted in structure **1**. C-33 was attached to C-27 on the basis of a long-range ^{1}H–^{1}H coupling observed between both H-33 and H-28 protons, and HMBC correlations between one of the H-33 protons and C-27 and C-28 and between H-28 and C-33 (Fig. 2). COSY and 1D TOCSY correlations irradiating the olefinic protons H-35 and H-36 established the sequence from C-33 to C-42, and the chemical shift of C-42 (δ_C 62.9 ppm), and HMBC cross-peaks between both H-42 protons and C-20 determined the linkage of C-42 to N-21. Finally, the C-43 methyl group was placed at C-41 on the basis of a ^{1}H–^{1}H coupling between H-43 and H-41 and HMBC correlations from H-40 and H-42 to C-43 and from H-43 to C-40, C-41, and C-42. As protons H-35 and H-36 were overlapped and it was therefore impossible to measure their coupling constant, the *Z* stereochemistry of the $\Delta^{35,36}$ double bond was assigned on the basis of the high field chemical shifts of C-34 and C-37 (δ_C 24.0 and 28.0 ppm, respectively).[3b,4,10]

Compound **2** had a molecular formula of $C_{41}H_{56}N_4O_2$, according to the (+)-HRMALDIMS (*m*/*z* [M+H]$^+$ 639.4648, calcd for $C_{41}H_{59}N_4O_2$, 639.4632) and ^{13}C NMR spectra, revealing the presence of an additional carbon with respect to

1. The most evident difference between the ^{1}H NMR spectra of both compounds (Table 1) was the presence of an additional methyl group at δ_H 1.12 (d, 6.5 Hz) in **2**, consistent with the difference observed in the molecular formula of the compounds. This methyl group (C-44) was placed at C-15 on the basis of a ^{1}H–^{1}H coupling between H-44 and H-15 and HMBC correlations observed between H-44 and C-14, C-15 and C-16. Carbons C-14, C-15, and C-16 were shifted slightly downfield whereas carbon C-13 was shielded with respect to compound **1** due to the presence of the new methyl group at C-15. In addition to the high field chemical shifts observed for carbons C-34 and C-37 (δ_C 23.8 and 27.5 ppm, respectively), the *Z* stereochemistry of the $\Delta^{35,36}$ double bond was also supported in the case of njaoamine B (**2**) by a NOESY correlation observed between one of the H-34 protons at δ_H 2.24 ppm and the H-37 proton resonating at δ_H 2.12 ppm.

The third compound in the series, njaoamine C (**3**), was isolated as an optically active yellowish gum of molecular formula $C_{39}H_{54}N_4O_2$, according to the (+)-HRMALDIMS and ^{13}C NMR spectra. A direct comparison of the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of this compound with those of njaoamines A and B revealed two major differences: the lack of methyl groups in **3** and the absence of the oxygenated methine present in the structures of **1** and **2**. These two findings were in agreement with the molecular formula observed for **3**. ^{1}H and ^{13}C chemical shifts and multiplicity patterns were almost identical from N-1 to C-32 in the structures of **1** and **3**. As in the case of njaoamine A, the length of the two carbon chains linking C-2 to N-17 and C-3 to C-19 was secured by 1D TOCSY, COSY, and HMBC experiments. The major differences were found in the carbon chain connecting C-27 with N-21 due to the absence of the hydroxyl and methyl groups present in structure **1**, with njaoamine C therefore having the structure depicted in **3**. Assignments of the chain from C-33 to C-42 were made with the help of COSY and HMBC experiments and comparison with the chemical shifts observed in **1** and **2**.

Njaoamine D (**4**) gave a parent ion in the (+)-HRMALDI mass spectrum at 609.4550 ([M+H]$^+$) consistent with a molecular formula of $C_{40}H_{56}N_4O$. This molecular formula and a comparison of the ^{1}H and ^{13}C NMR spectra with those of njaoamine A revealed that the only difference between the compounds was the absence of the oxygenated methine present at C-40 in **1**. C-39, C-40, and C-41 carbons were consequently shifted upfield in **4** by 10.2, 40.8, and 6.5 ppm, respectively.

Compound **5** had a molecular formula of $C_{41}H_{54}N_4O$ according to the (+)-HRMALDIMS (*m*/*z* [M+H]$^+$ 619.4383 calcd for $C_{41}H_{55}N_4O$, 619.4370) and ^{13}C NMR spectra, having therefore 17 degrees of unsaturation. A careful analysis of the 1D (Tables 2 and 3) and 2D NMR spectra of this compound revealed the presence of the same substructure from N-1 to C-32 as present in njaoamines A, C, and D. The major differences were found in the carbon bridge between C-27 and N-21. This bridge contained six aliphatic methylenes and six olefinic methine carbons, consistent with the presence of a linear chain containing three alkene functionalities. HMBC, HSQC, and COSY spectra located the position of the alkenes at $\Delta^{35,36}$, $\Delta^{38,39}$, and $\Delta^{41,42}$. The high field ^{13}C NMR chemical shifts of the C-34 (δ_C 24.7), C-37 (25.7), C-40 (26.2), and C-43 (22.2) ppm allylic carbons together with the ^{1}H–^{1}H olefinic coupling constants measured for protons H-36 (10.4 Hz) and H-41 (10.1 Hz) were consistent with the *Z* configuration for all the three alkenes.

The last compound of the series, njaoamine F (**6**), had a molecular formula of $C_{43}H_{56}N_4O$, based on a pseudomolecular ion at *m*/*z* 645.4528 in its (+)-HRMALDI mass spectrum (calcd for $C_{43}H_{57}N_4O$, 645.4527). As in the case of njaoamine E (**5**), the major differences between **6** and rest of the compounds were found in the linear carbon bridge from C-27 to N-21. Six aliphatic methylenes and eight olefinic methine carbons (C-33–C-46) were identified as the constituents of this bridge. HMBC, HSQC, and COSY spectra located the position of the alkenes at $\Delta^{34,35}$, $\Delta^{37,38}$, $\Delta^{40,41}$, and $\Delta^{43,44}$. Coupling constants measured for the olefinic protons H-34 (10.5 Hz), H-37 and H-38 (11.0 Hz), and H-43 (10.6 Hz), together with the chemical shifts measured for carbons C-36 (δ_C 25.7), C-39 (26.1), C-42 (26.1), and C-44 (22.6) ppm indicated that all the four olefins in the bridge had the *Z* configuration.

Figure 3 represents a proposed biogenesis for the njaoamine group of alkaloids. The precursor **I**, obtained by

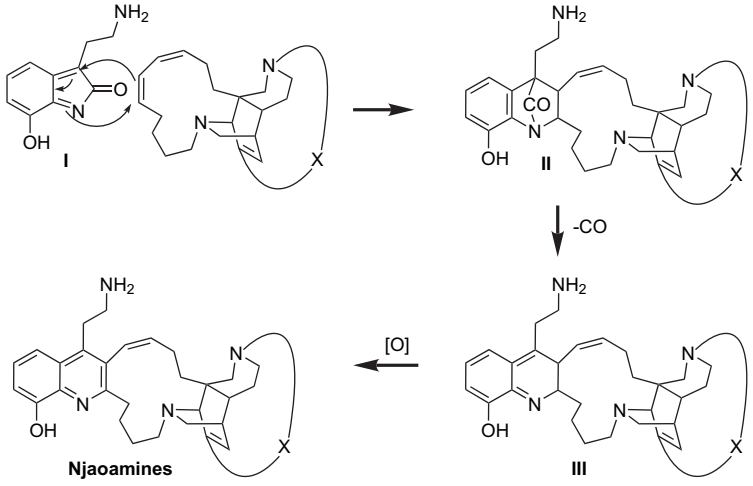

Figure 3. Proposed biogenesis for the njaoamine family of alkaloids.

decarboxylation and oxidation of tryptophan, would undergo a hetero Diels–Alder reaction with a pentacyclic ingenamine-like core to give adduct **II**. Loss of CO and final oxidation of intermediate **III** would produce the njaoamine family of compounds.

The cytotoxic activity of njaoamines A–F (**1**–**6**) was evaluated against a panel of three human tumor cell lines, including colon (H-T29), lung (A-549), and breast (MDA-MB-231).[11] All the six compounds isolated displayed cytotoxic properties, with GI_{50} values in the micromolar range (Table 4). No noticeable differences in activity were found among the different members of the family.

Table 4. GI_{50} values (μM) of compounds **1**–**6** against tumor cell lines

Compound	Cell lines		
	A-549	HT-29	MDA-MB-231
1	6.7	6.7	6.6
2	1.5	2.2	1.5
3	6.6	6.4	5.2
4	7.2	6.7	7.2
5	3.0	3.7	2.8
6	5.7	6.1	6.7

3. Conclusion

In conclusion, six members of a new family of alkaloids, all with cytotoxic properties, have been isolated from the sponge *Reniera* sp. The choice of the appropriate NMR solvent was crucial in the structural determination of this family of complex polycyclic alkaloids. These results are further evidence that sponges of the order Haplosclerida are a rich source of alkylpyridine derived alkaloids and that these compounds may be used as chemotaxonomic markers of the order. Whilst being ingenamine-related metabolites, the novel compounds reported herein all contain an 8-hydroxyquinoline moiety, an element of structural novelty without precedent in other members of the family. Our findings also further prove that many marine organisms produce structurally novel chemical entities that may be lethal to cancer cells and are therefore potential new drugs for the treatment of this disease.

4. Experimental section

4.1. General experimental procedures

Optical rotations were determined in MeOH using a Jasco P-1020 Polarimeter. UV spectra were obtained with a Perkin Elmer Lambda 15 UV/Vis Spectrophotometer. IR spectra were recorded on a Perkin Elmer 881 Infrared Spectrophotometer. NMR spectra were recorded on a Varian 'Unity 500' spectrometer at 500/125 MHz ($^1H/^{13}C$). Chemical shifts were reported in parts per million using residual non-deuterated pyridine signals (δ 7.19 ppm for ^1H and 123.5 ppm for ^{13}C) as internal reference. Accurate mass analyses were performed by (+)-HRMALDIMS on an Applied Biosystems 4700 Proteomic Analyzer employing 2,5-dihydroxybenzoic acid (DHB) as the matrix.

4.2. Sponge collection and identification

Samples of *Reniera* sp. were collected in December 2004 by hand using scuba at Njao (Tanzania), at depths ranging from 7 to 38 m, and kept frozen until used. The material was identified by Dr. José Luis Carballo from the University Autónoma de México (México). A voucher specimen (ORMA033212) is deposited at PharmaMar.

4.3. Extraction and isolation

Frozen specimens of the sponge (803 g) were triturated and exhaustively extracted with 2-propanol (3×1 L). The extract was concentrated under vacuum to yield a crude of weight 84 g. This crude material was resuspended in H_2O–MeOH (4:1) (500 mL) and partitioned between *n*-hexane (3× 500 mL), EtOAc (3×500 mL), and *n*-BuOH (2×500 mL). The *n*-BuOH extract (8.2 g) was subjected to VLC over RP-18 silica gel with a stepped gradient from H_2O to MeOH. Fractions eluted with MeOH–H_2O (3:1) (1.96 g) were subjected to preparative HPLC (Symmetry C18, 19× 150 mm, gradient H_2O+0.1% TFA–CH_3CN+0.1% TFA from 10 to 23% of CH_3CN in 35 min, 13.6 mL/min, UV detection) to yield five active fractions (F1–F5). Fraction F1 was purified by preparative HPLC (Symmetry C18, 19× 150 mm, gradient H_2O+0.1% TFA–CH_3CN+0.1% TFA from 15 to 20% of CH_3CN in 30 min, 13.6 mL/min, UV detection) to yield 57 mg of pure **1**. Compounds **2** (2.0 mg), **3** (14.0 mg), and **4** (13.0 mg) were obtained from fraction F3 by preparative HPLC (Symmetry C18, 19×150 mm, gradient H_2O+0.1% TFA–CH_3CN+0.1% TFA from 35 to 45% of CH_3CN in 30 min, 13.6 mL/min, UV detection) followed by semipreparative HPLC (Symmetry C18, 7.8×150 mm, gradient H_2O+0.1% TFA–CH_3CN+0.1% TFA from 35 to 45% of CH_3CN in 30 min, 2.3 mL/min, UV detection). Finally, preparative HPLC (Symmetry C18, 19×150 mm, gradient H_2O+0.1% TFA–CH_3CN+0.1% TFA from 35 to 45% of CH_3CN in 30 min, 13.6 mL/min, UV detection) of fraction F4 afforded compounds **5** (2.4 mg) and **6** (12.0 mg).

4.3.1. Njaoamine A (1). Yellowish gum; $[\alpha]_D^{25}$ +61.2 (*c* 0.11, MeOH); UV (MeOH) λ_{max} 204, 252, 314 nm; IR (KBr) ν_{max} 3430, 2945, 1677, 1531, 1400, 1292, 1200, 1133, 831, 798, 750, 720, 706 cm^{-1}; ^1H and ^{13}C NMR: see Tables 1 and 2; (+)-HRMALDIMS *m/z* 625.4479 $[M+H]^+$ (calcd for $C_{40}H_{57}N_4O_2$, 625.4476, Δ 0.4 ppm).

4.3.2. Njaoamine B (2). Yellowish gum; $[\alpha]_D^{25}$ +51.1 (*c* 0.11, MeOH); UV (MeOH) λ_{max} 204, 252, 312 nm; IR (KBr) ν_{max} 3421, 2944, 1678, 1527, 1423, 1356, 1310, 1197, 1131, 834, 798, 721 cm^{-1}; ^1H and ^{13}C NMR: see Tables 1 and 2; (+)-HRMALDIMS *m/z* 639.4648 $[M+H]^+$ (calcd for $C_{41}H_{59}N_4O_2$, 639.4632, Δ 2.5 ppm).

4.3.3. Njaoamine C (3). Yellowish gum; $[\alpha]_D^{25}$ +65.7 (*c* 0.6, MeOH); UV (MeOH) λ_{max} 204, 252, 313 nm; IR (KBr) ν_{max} 3439, 1678, 1435, 1205, 1132, 837, 801, 724 cm^{-1}; ^1H and ^{13}C NMR: see Tables 1 and 2; (+)-HRMALDIMS *m/z* 595.4396 $[M+H]^+$ (calcd for $C_{39}H_{55}N_4O_2$, 595.4370, Δ 4.4 ppm).

4.3.4. Njaoamine D (4). Yellowish gum; $[\alpha]_D^{25}$ +51.6 (*c* 0.7, MeOH); UV (MeOH) λ_{max} 204, 251, 312 nm; IR

(KBr) ν_{max} 3435, 2941, 1675, 1427, 1201, 1133, 836, 799, 740, 722 cm^{-1}; ^1H and ^{13}C NMR: see Tables 1 and 2; (+)-HRMALDIMS *m/z* 609.4550 $[M+H]^+$ (calcd for $C_{40}H_{57}N_4O$, 609.4527, Δ 3.8 ppm).

4.3.5. Njaoamine E (5). Yellowish gum; $[\alpha]_D^{25}$ +24.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 204, 252 nm; IR (KBr) ν_{max} 3439, 1676, 1435, 1202, 1132, 799, 722 cm^{-1}; ^1H and ^{13}C NMR: see Tables 3 and 2, respectively; (+)-HRMALDIMS *m/z* 619.4383 $[M+H]^+$ (calcd for $C_{41}H_{55}N_4O$, 619.4370, Δ 2.0 ppm).

4.3.6. Njaoamine F (6). Yellowish gum; $[\alpha]_D^{25}$ +20.1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 204, 252 nm; IR (KBr) ν_{max} 3434, 2933, 1673, 1429, 1203, 1131, 800, 722 cm^{-1}; ^1H and ^{13}C NMR: see Tables 3 and 2, respectively; (+)-HRMALDIMS *m/z* 645.4528 $[M+H]^+$ (calcd for $C_{43}H_{57}N_4O$, 645.4527, Δ 0.2 ppm).

4.4. Cytotoxicity assay

A-549 (ATCC CCL-185), lung carcinoma; HT-29 (ATCC HTB-38), colorectal carcinoma; and MDA-MB-231 (ATCC HTB-26), breast adenocarcinoma cell lines were obtained from the ATCC. Cell lines were maintained in RPMI medium supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, and 100 U/mL penicillin and streptomycin, at 37 °C and 5% CO_2. Triplicate cultures were incubated for 72 h in the presence or absence of test compounds (at 10 concentrations ranging from 10 to 0.0026 μg/mL). For quantitative estimation of cytotoxicity, the colorimetric sulforhodamine B (SRB) method was used, essentially performed as described previously.[11] Briefly, cells were washed twice with PBS, fixed for 15 min in 1% glutaraldehyde solution, rinsed twice in PBS, and stained in 0.4% SRB solution for 30 min at room temperature. Cells were then rinsed several times with 1% acetic acid solution and air-dried. Sulforhodamine B was then extracted in 10 mM trizma base solution and the absorbance measured at 490 nm. Results are expressed as GI_{50}, the concentration that causes 50% inhibition in cell growth after correction for cell count at the start of the experiment (NCI algorithm).

Acknowledgements

The authors thank Dr. José Luis Carballo for the taxonomic identification of the sponge and Dr. Luis F. García-Fernández for performing the cytotoxicity assays.

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