



**HAL**  
open science

## Isolation and structure elucidation of cyclopeptide alkaloids from the leaves of *Heisteria parvifolia*

Michel Boni Bitchi, Abdulmagid Alabdul-Magid, Faustin Aka Kabran, Philomène Akoua Yao-Kouassi, Dominique Harakat, Hamid Morjani, Félix Zanahi Tonzibo, Laurence Voutquenne-Nazabadioko

### ► To cite this version:

Michel Boni Bitchi, Abdulmagid Alabdul-Magid, Faustin Aka Kabran, Philomène Akoua Yao-Kouassi, Dominique Harakat, et al.. Isolation and structure elucidation of cyclopeptide alkaloids from the leaves of *Heisteria parvifolia*. *Phytochemistry*, 2019, 167, pp.112081. 10.1016/j.phytochem.2019.112081 . hal-02310316v2

**HAL Id: hal-02310316**

**<https://hal.univ-reims.fr/hal-02310316v2>**

Submitted on 24 Sep 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Isolation and structure elucidation of cyclopeptide alkaloids from the leaves of *Heisteria***  
2 ***parvifolia***  
3

4 Michel Boni Bitchi<sup>a,b</sup>, Abdulmagid Alabdul Magid<sup>a</sup>, Faustin Aka Kabran<sup>b</sup>, Philomène Akoua  
5 Yao-Kouassi<sup>b</sup>, Dominique Harakat<sup>a</sup>, Hamid Morjani<sup>c</sup>, Félix Zanahi Tonzibo<sup>b,\*</sup>, Laurence  
6 Voutquenne-Nazabadioko<sup>a</sup>

7 <sup>a</sup>Université de Reims Champagne Ardenne, CNRS, ICMR UMR 7312, 51097 Reims, France

8 <sup>b</sup>Laboratoire de Chimie Organique Biologique, UFR Sciences des Structures de la Matière et  
9 Technologie, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Cote d'Ivoire

10 <sup>c</sup>Université de Reims Champagne Ardenne, BioSpect EA 7506, 51097 Reims, France

11

12

13

14

15

16

17

18 **\*Corresponding authors.** Tel: +225 05 07 19 67

19 E-mail address: [tonzibz@yahoo.fr](mailto:tonzibz@yahoo.fr) (F.Z. Tonzibo), [abdulmagid.alabdulmagid@univ-reims.fr](mailto:abdulmagid.alabdulmagid@univ-reims.fr)

20 (A. Alabdul Magid)

21

22

23 **Highlights**

- 24 ► Five undescribed cyclopeptide alkaloids were isolated from *Heisteria parvifolia* Sm.  
25 ► Their structures were elucidated by 1D-, 2D-NMR and HR-ESI-MS analyses.  
26 ► Their cytotoxicity against the chronic myeloid leukemia K562 cells was evaluated.

27

28

29 **ABSTRACT**

30 *Heisteria parvifolia* Sm. is prescribed in traditional medicine against numerous diseases in Côte  
31 d'Ivoire. Due to the shortcoming in scientific knowledge of use of this species, our  
32 investigations revealed five undescribed cyclopeptide alkaloids added to one known derivative  
33 namely anorldianine. These compounds were elucidated by 1D- and 2D-NMR experiments and  
34 comparison with literature data, and confirmed by HR-ESI-MS. Cytotoxic activity evaluation  
35 of these compounds against the chronic myeloid leukemia (K565) cell line exhibited an  
36 antiproliferative activity with cell growth inhibition from 13% to 46%

37

38 **Keyword:** *Heisteria parvifolia*; Olacaceae; cyclopeptide alkaloids; cytotoxic activity; chronic  
39 myeloid leukemia (K565) cell line

40

## 41 1. Introduction

42 Cyclopeptide alkaloids are widespread and occur in several families: Asteraceae, Celastraceae,  
43 Euphorbiaceae, Fabaceae, Menispermaceae, Olacaceae, Pandaceae, Rhamnaceae, Rubiaceae,  
44 Sterculiaceae, and Urticaceae (El-Seedi et al. 2007, Gournelis et al. 1997, Morel et al. 2009,  
45 Tan and Zhou, 2006). Previous studies have reported cyclopeptides alkaloids from *Heisteria*  
46 *nitida* (El-Seedi et al. 1999, El-Seedi et al. 2005). The cyclopeptide alkaloids *sensu stricto* were  
47 classified according to the number of amino acid constituents outside and the size of the  
48 macrocycle (inside) as 4(13); 5(13); 4(14) and 4(15) type of alkaloids (Joullie and Richard,  
49 2004, Tan and Zhou, 2006). Several activities of cyclopeptides alkaloids have been reported,  
50 such as antimicrobial (Gournelis et al. 1997, Morel et al. 2005), insecticidal (Sugawara et al.  
51 1996), sedative (Suh et al. 1997), and antiplasmodial activity (Suksamrarn et al. 2005).

52 The genus *Heisteria* belonging to the *Olacaceae* family comprises about 65 species in tropical  
53 America and 3 in Africa; namely *Heisteria parvifolia* Sm., *Heisteria trillesiana* Pierre ex  
54 Heckel, and *Heisteria zimmereri* Engl. *Heisteria parvifolia* Sm. is an evergreen shrub or small  
55 tree up to 15 (-20) m tall; 40 (-60) cm in diameter (Malaisse et al. 2004). *H. parvifolia* occurs  
56 from Senegal and south-western Mali eastward to the Central African Republic and southward  
57 DR Congo and northern Angola; possibly also in Uganda and southern Soudan (Louppe et al.  
58 2008). In Côte d'Ivoire, is locally abundant on sandy soils. Its wood is used for construction and  
59 tool handles. In several areas, the fruits are eaten fresh; the small oil-rich seeds are eaten fresh,  
60 roasted or cooked. The twigs are used as chew-sticks. Various *Heisteria* species are used by  
61 South-American Indians or in Africa in the treatment of rheumatism, abscesses, headache,  
62 throat infections, swellings, nose bleedings, pain in joints and muscles, diarrhea, hepatic  
63 infection (Kvist and Holm-Nielsen, 1987, Russo, 1992, Tan and Zhou, 2006). In traditional  
64 medicine in Ghana, ground roots of *H. parvifolia* are applied as enema against stomach-ache.  
65 In Congo, sap from the root bark is used as dropps into the nose against migraine and into the  
66 eye to treat painful, infected eyes. Stem bark is taken in Ghana, in Côte d'Ivoire and DR Congo  
67 as cough medicine. In Gabon, bark is applied to circumcision wounds. In Ghana and Côte  
68 d'Ivoire, leaf decoctions are taken or applied as a bath to invigorate rachitic children and to treat  
69 convulsions. They are also used as analgesic and rubbed onto painful breasts of young mothers,  
70 and in Sierra Leone to treat tooth-ache. In Congo, leaf decoctions are administrated against  
71 asthma, costal pain, stomach pain, and menstrual problems. Ground seeds are used to stupefy  
72 fish. In DR Congo, powdered bark is an ingredient in the preparation of arrow poison (Abbiw  
73 1990, Burkill 1997, Malaisse et al. 2004). Chemical investigations of *Heisteria* species have  
74 mainly revealed the presence of triterpenes and proanthocyanidines in *H. pallida* (Dirsch et al.

75 1992, Dirsch et al. 1993), cyclopeptide alkaloid in *H. nitida* (El-Seedi et al. 1999, El-Seedi et  
76 al. 2005), scopolamine in *H. olivae* (Cairo-Valera et al. 1977), and acetylenic fatty acids in *H.*  
77 *accuminata* (Kraus et al. 1998). Up to date, only the composition of the seeds oil of *H. parvifolia*  
78 has been reported as mainly long-chain saturated fatty acids, oleic acid and other mono and di  
79 enoic fatty acids (Malaisse et al. 2004).

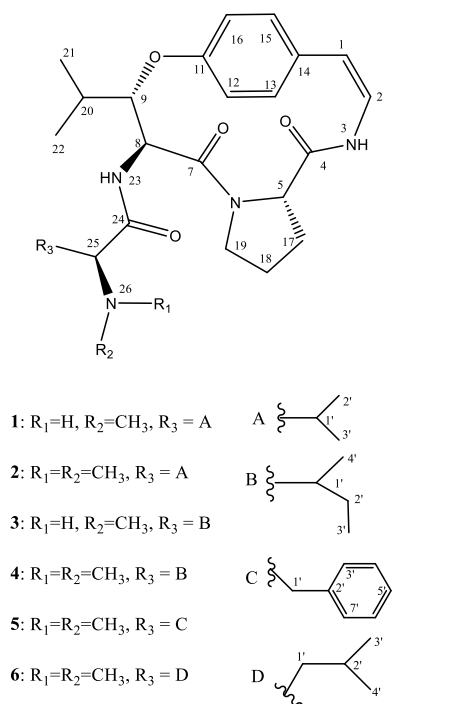
80 As a part of a continuing study for the discovery of medicinal Côte d'Ivoire species, five  
81 undescribed cyclopeptide alkaloids (**1-5**), together with one known compound (**6**), have been  
82 isolated and characterized from the leaves of *H. parvifolia*. Their cytotoxicity against the  
83 chronic myeloid leukemia K562 cells was evaluated.

84

## 85 2. Results and discussion

86 The crude alkaloid extract prepared with an acid-base method of air-dried and pulverized leaves  
87 of *H. parvifolia* was subjected to silica gel flash chromatography, eluted with increasingly polar  
88 mixtures of CHCl<sub>3</sub>/MeOH. Further purification was performed using semi-preparative HPLC.  
89 As a result, five undescribed cyclopeptide alkaloids (**1-5**) were isolated and chemically  
90 characterized, together with one known cyclopeptide alkaloids, anorldianine (**6**) (El-Seedi et al.  
91 1999). Their structures (Fig. 1) were elucidated by 1D- and 2D-NMR experiments and  
92 comparison with literature data, and confirmed by HR-ESI-MS.

93



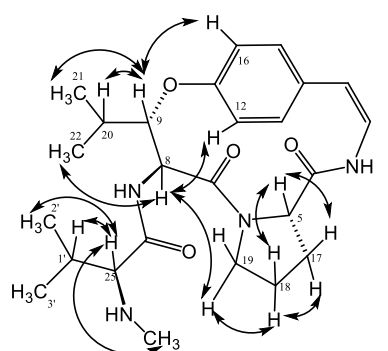
94

95 Fig. 1. Chemical structures of compounds **1-6**.

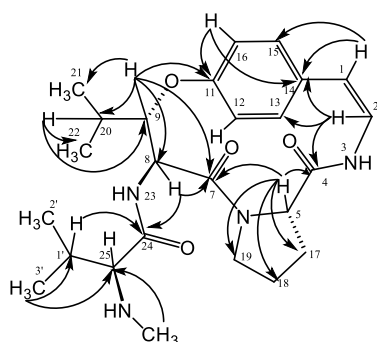
96 The UV spectra of compounds **1-5** showed absorptions at 222-224 and 274-282 nm,  
97 wavelengths commonly assigned to peptide bonds and aromatic residues (Dongo et al. 1989;  
98 Kang et al. 2015, Schwing et al. 2011), while their IR spectra displayed bands at 3395 and 1682  
99  $\text{cm}^{-1}$ , which are typical of amide groups (Dongo et al. 1989, Schwing et al. 2011).

100 Compound **1** was obtained as a white powder. Analysis of **1** by high-resolution  
101 electrospray ionization mass spectrometry (HR-ESI-MS) identified a pseudo-molecular ion  $[\text{M}$   
102  $+ \text{H}]^+$  at  $m/z$  457.2807, corresponding to the molecular formula  $\text{C}_{25}\text{H}_{36}\text{N}_4\text{O}_4$  (calcd for  
103  $\text{C}_{25}\text{H}_{37}\text{N}_4\text{O}_4$ , 457.2815), in combination with analysis of NMR data. The  $^{13}\text{C}$  NMR (Table 1)  
104 and HSQC spectra of **1** showed 25 carbon resonances for four methyls ( $\delta_{\text{C}}$  13.9, 16.7, 17.5, and  
105 19.5), one *N*-methyl ( $\delta_{\text{C}}$  31.8), three methylenes ( $\delta_{\text{C}}$  23.3, 28.2, and 46.7), twelve methines (two  
106 of which were olefinic carbons at  $\delta_{\text{C}}$  116.7 and 124.9 and four were aromatic carbons  $sp^2$  at  $\delta_{\text{C}}$   
107 120.9, 121.9, 131.0, and 131.4), two quaternary aromatic carbons ( $\delta_{\text{C}}$  157.2 and 131.6), and  
108 three carbonyl carbons ( $\delta_{\text{C}}$  171.1, 167.5, and 165.2). The  $^1\text{H}$  NMR spectrum (Table 1) displayed  
109 signals for two olefinic protons at  $\delta_{\text{H}}$  6.53 and 6.65, a singlet *N*-methyl ( $\delta_{\text{H}}$  2.68), four methyl  
110 doublets, four aromatic protons, and several methine and methylene protons. The NMR data of  
111 compound **1** (Table 1) showed great similarity with the NMR data previously reported for  
112 anorldianine (compound **6**) possessing a 14-membered ring type comprising a *p*-oxygenated *Z*-  
113 styrylamine group (Dongo et al. 1989, El-Seedi et al. 1999, El-Seedi et al. 2005). The presence  
114 of the *p*-oxygenated *Z*-styrylamine group was indicated by two doublets at  $\delta_{\text{H}}$  6.53 and 6.65  
115 (each 1H,  $J = 7.7$  Hz; H-1 and H-2, respectively) corresponding to the *Z*-double bond and four  
116 aromatic protons appearing as doublets of doublets with *J*-values typical for an *o,m*-coupling  
117 pattern (H-12, 13, 15, and 16). The protons H-13 and H-15 ( $\delta_{\text{H}}$  7.11, dd,  $J = 8.7, 2.4$  Hz) showed  
118 correlation with C-1 ( $\delta_{\text{C}}$  116.7) and H-12 and H-16 ( $\delta_{\text{H}}$  7.27, dd,  $J = 8.7, 2.4$  Hz) with C-14 ( $\delta_{\text{C}}$   
119 131.6) in HMBC spectrum. In cyclopeptide alkaloids, the H-9 ( $\beta$ -H of the  $\beta$ -hydroxy-amino  
120 acid moiety) chemical shift value (between 5.00 and 5.50 ppm) is characteristic. In this case, a  
121 doublets of doublets was present at  $\delta_{\text{H}}$  4.92 ( $J = 8.3, 1.5$  Hz). In the COSY spectrum, two cross  
122 peaks were observed for H-9 ( $\delta_{\text{H}}$  4.92), more specifically with H-8 ( $\delta_{\text{H}}$  5.01, d,  $J = 8.3$  Hz) and  
123 H-20 ( $\delta_{\text{H}}$  2.13, sept,  $J = 6.9$  Hz). The proton signal of the CH-group in position 20 also showed  
124 cross peaks to H<sub>3</sub>-21 ( $\delta_{\text{H}}$  1.32, d,  $J = 6.7$  Hz) and H<sub>3</sub>-22 ( $\delta_{\text{H}}$  1.05, d,  $J = 6.7$  Hz). In the HMBC  
125 spectrum, correlations between C-9 ( $\delta_{\text{C}}$  83.4) and H-21 and H-22 were observed (Fig. 2). These  
126 data agreed with previously reported data for  $\beta$ -hydroxyleucine (anorldianine). The methine  
127 and the methylene protons of proline were observed in the  $^1\text{H}$  NMR spectrum: H-5 ( $\delta_{\text{H}}$  4.16,  
128 dd,  $J = 7.5, 1.9$  Hz), H<sub>2</sub>-17 ( $\delta_{\text{H}}$  1.65, m; 2.21, dd,  $J = 11.5-4.5$  Hz), H<sub>2</sub>-18 ( $\delta_{\text{H}}$  1.75, m; 1.95, m),

129 and H<sub>2</sub>-19 ( $\delta_{\text{H}}$  3.55, brt,  $J = 9.8$  Hz; 3.85, m). In the COSY spectrum, cross peaks were observed  
 130 between H-5/H-17, H-17/H-18, and H-18/H-19. In the HMBC spectrum, the H-5 exhibited  
 131 correlations with C-19 ( $\delta_{\text{C}}$  46.7) and with the carbonyl C-4 ( $\delta_{\text{C}}$  167.5) (Fig. 2). The methyl  
 132 doublets of *N*-methyl-valine (H-2' and H-3') appeared at  $\delta_{\text{H}}$  0.95 ( $J = 6.7$  Hz) and 0.96 ( $J = 6.7$   
 133 Hz) and the methine proton (H-1') of this moiety appeared as septuplet at  $\delta_{\text{H}}$  2.13 ( $J = 6.9$  Hz).  
 134 The HMBC experiment showed correlations between C-24 ( $\delta$  165.2) and H-25 ( $\delta_{\text{H}}$  3.57, d,  $J =$   
 135 5.4 Hz) and H-1', and between the *N*-methyl carbon ( $\delta_{\text{C}}$  31.8) and the H-25 for this moiety. The  
 136 combined use of 1D (<sup>1</sup>H and <sup>13</sup>C NMR) and 2D (COSY, HSQC, and HMBC) spectra allowed  
 137 an unambiguous assignment of all protons and carbons of the amino acids units ( $\beta$ -  
 138 hydroxyleucine, proline, *N*-methyl-valine residues) and the *p*-oxygenated *Z*-styrylamine group  
 139 (Table 1). Moreover, the connectivity between the constitutive parts of the molecule were  
 140 ascertained by the HMBC correlations between the carbonyl C-4/H-2, the carbonyl C-7/H-5,  
 141 the carbonyl C-24/H-8, and C-11/H-9 (Fig. 2). Moreover, the NOE relationships H-8/H-25, H-  
 142 8/ H-12 and H-9/H-16 agreed with the  $\beta$ -hydroxyleucine connection with the *N*-methyl-valine  
 143 and the *p*-oxygenated *Z*-styrylamine moieties whereas the NOE effects between H-8/H-19  
 144 agreed with the connection of  $\beta$ -hydroxyleucine with proline (Fig. 2). Compound **1** was named  
 145 cycloheisterin A after its plant origin (Fig. 1).



↔ NOESY



↔ HMBC

146

147 Fig. 2. Selected key HMBC and NOESY interactions for compound **1**.

148 Compound **2** displayed an  $[M + H]^+$  ion peak at  $m/z$  471.2979 in the positive HR-ESI-  
149 MS, corresponding to the molecular formula  $C_{26}H_{38}N_4O_4$ , suggesting an additional methyl  
150 group compared to **1**. The NMR spectroscopic data of **2** were almost identical with those of **1**  
151 except for one additional methyl group (Table 1). The detailed analysis of the 2D-NMR spectra  
152 led to the identification, as in **1**, of the amino acids units ( $\beta$ -hydroxyleucine, proline, and valine  
153 residues) and the *p*-oxygenated *Z*-styrylamine group (Table 1). The HMBC cross-peaks of the  
154 methyl signals at  $\delta_H$  2.92 (6H, s) to C-25 ( $\delta_C$  72.7) of the valine residue indicated that the  
155 terminal amino acid in **2** is *N,N*-dimethyl-valine. Compound **2** was named cycloheisterin B.

156 Compound **3** displayed an  $[M + Na]^+$  ion peak at  $m/z$  493.2785 in the positive HR-ESI-  
157 MS, corresponding to the molecular formula  $C_{26}H_{38}N_4O_4$ , suggesting an additional methylene  
158 group compared to **1**. The  $^1H$  and  $^{13}C$  NMR values of **3** were almost superimposable on those  
159 of **1** (Table 1) excepting those of the *N*-methyl-valine residue in **1**. Instead, an *N*-methyl-  
160 isoleucine residue was identified as summarized in Table 1 (Tuenter et al. 2017).  $^1H$ - $^1H$  COSY  
161 analysis confirmed the presence of an isoleucine residue. The HMBC cross-peak of H-8 of  
162 hydroxyleucine ( $\delta_{H-8}$  5.01) to C-24 of isoleucine residue ( $\delta_{C-24}$  165.3) and H-25 ( $\delta_{H-25}$  3.62) to  
163 C-24 and the *N*-methyl carbon ( $\delta_C$  31.8) to H-25 for this moiety confirmed that the terminal  
164 amino acid is *N*-methyl-isoleucine. Compound **3** was named cycloheisterin C (Fig. 1).

165 Cycloheisterin D (**4**) displayed an  $[M + H]^+$  ion peak at  $m/z$  485.3138 in the positive  
166 HR-ESI-MS, corresponding to the molecular formula  $C_{27}H_{40}N_4O_4$ , suggesting an additional  
167 methyl group compared to **3**. Comparing the NMR data of **4** with those of **3** (Table 1) and the  
168 analysis of the 2D-NMR spectra led to the identification, as in **3**, of the amino acids units ( $\beta$ -  
169 hydroxyleucine, proline and isoleucine residues) and the *p*-oxygenated *Z*-styrylamine group  
170 (Table 1). The HMBC cross-peaks of the methyl signals at  $\delta_H$  2.91 (6H, s) to C-25 ( $\delta_C$  72.2) of  
171 the isoleucine residue suggested that the terminal amino acid in **4** is *N,N*-dimethyl-isoleucine.  
172 Compound **4** was named cycloheisterin D (Fig. 1).

173 Cycloheisterin E (**5**) exhibited an  $[M + Na]^+$  ion peak at  $m/z$  541.2799 in the HR-ESI-  
174 MS spectrum, consistent with the molecular formula of  $C_{30}H_{38}N_4O_4$ . Comparing the NMR data  
175 of **5** with those of **1-4** (Table 1) and detailed analysis of the 2D-NMR spectra showed that it  
176 had the same macrocycle (inside) composed of the amino acids units ( $\beta$ -hydroxyleucine and  
177 proline) and the *p*-oxygenated *Z*-styrylamine group (Table 1). The  $^1H$  and  $^{13}C$ -NMR spectra of  
178 **5** exhibited signals corresponding to an aromatic amino acid [ $\delta_H$  7.20-7.30, 5H]. Extensive 2D-  
179 NMR analysis enabled the full assignments of the *N,N*-dimethyl phenylalanine. (Tuenter et al.  
180 2017). The presence of the *N,N*-dimethyl groups was confirmed by the HMBC correlation



181 between the methyl signals at  $\delta_{\text{H}}$  2.97 (6H, s) and C-25 ( $\delta_{\text{C}}$  68.1) of the phenylalanine residue.  
 182 The HMBC correlation between H-8 ( $\delta_{\text{H}}$  4.82) of the  $\beta$ -hydroxyleucine and the C-24 ( $\delta_{\text{C}}$  165.0)  
 183 of the *N,N*-dimethyl phenylalanine confirmed it to be the terminal amino acid moiety.  
 184 Compound **5** was named cycloheisterin E (Fig. 1).

185 **Table 1.**  $^{13}\text{C}$  NMR spectroscopic data for compounds **1-5** (500 MHz,  $\text{CD}_3\text{OD}$ ).

	1		2		3		4		5	
	$\delta_{\text{H}}$ m (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ m (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ m (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ m (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ m (J in Hz)	$\delta_{\text{C}}$
<b>1</b>	6.53, d (7.7)	116.7	6.53, d (7.7)	116.8	6.53, d (7.7)	116.3	6.53, d (7.7)	116.8	6.50, d (7.7)	116.8
<b>2</b>	6.65, d (7.7)	124.9	6.65, d (7.7)	125.0	6.66, d (7.7)	124.9	6.65, d (7.7)	124.9	6.60, d (7.7)	125.0
<b>4</b>	-	167.5	-	167.5	-	167.5	-	167.4	-	167.9
<b>5</b>	4.16, dd (7.5, 1.9)	62.5	4.14, d (7.5)	62.6	4.15, d (7.8)	62.5	4.12, d (8.1)	62.6	3.72, d (8.0)	62.4
<b>7</b>	-	171.1	-	171.0	-	171.1	-	171.0	-	170.7
<b>8</b>	5.01, d (8.3)	53.1	5.01, d (8.5)	52.0	5.01, d (8.3)	53.1	5.01, d (8.5)	53.0	4.82, (overlapped)	53.0
<b>9</b>	4.92, dd (8.3, 1.5)	83.4	4.93, dd (8.5, 1.9)	83.2	4.91, dd (8.3, 1.2)	83.4	4.92, dd (8.3, 1.3)	83.2	4.82, (overlapped)	82.7
<b>11</b>	-	157.2	-	157.2	-	157.2	-	157.2	-	157.1
<b>12</b>	7.27, dd (8.7, 2.4)	120.9	7.27, d (8.5)	120.8	7.27, d (8.1)	120.9	7.27, d (8.9)	120.8	7.20, m	119.6
<b>13</b>	7.11, dd (8.7, 2.4)	131.4	7.11, m	131.4	7.11, m	131.4	7.11, m	131.4	7.11, dd (8.5, 1.5)	131.3
<b>14</b>	-	131.6	-	131.8	-	131.6	-	131.6	-	131.3
<b>15</b>	7.11, dd (8.7, 2.4)	131.0	7.11, m	130.3	7.11, m	130.5	7.11, m	130.3	7.12, dd (8.5, 1.5)	130.2
<b>16</b>	7.27, dd (8.7, 2.4)	120.9	7.27, d (8.5)	120.8	7.27, d (8.1)	120.9	7.27, d (8.9)	120.8	7.20, m	120.3
<b>17</b>	1.65, m	28.2	1.67, m	28.2	1.65, m	28.1	1.64, m	28.2	1.54, m	28.6
	2.21, dd (11.5, 4.5)		2.21, dd (12.3, 4.9)		2.21, dd (12.1, 5.8)		2.22, dd (12.5, 6.1)		2.01, dd (11.3, 4.8)	
<b>18</b>	1.75, m	23.3	1.75, m	23.3	1.75, m	23.3	1.75, m	23.3	1.67, m	23.5
	1.95, m		1.97, m		1.95, m		1.97, m		1.82, m	
<b>19</b>	3.55, brt (9.8)	46.7	3.55, brt (10.8)	46.7	3.55, brt (9.8)	46.7	3.58, brt (9.3)	46.8	3.42, brt (9.2)	47.0
	3.85, m		3.90, ddd (10.8, 7.1, 3.2)		3.85, m		3.85, ddd (10.1, 9.3, 7.4)		3.71, m	
<b>20</b>	2.13, m	28.7	2.12, dq (6.8, 1.7)	28.7	2.13, m	28.7	2.11, m	28.7	2.10, m	28.6
<b>21</b>	1.32, d (6.7)	19.5	1.32, d (6.8)	19.4	1.32, d (6.8)	19.5	1.32, d (6.8)	19.4	1.27, d (6.8)	19.3
<b>22</b>	1.05, d (6.7)	13.9	1.07, d (6.8)	14.0	1.06, d (6.8)	13.9	1.07, d (6.8)	14.0	1.02, d (6.8)	14.0
<b>24</b>	-	165.2	-	164.3	-	165.3	-	164.4	-	165.0
<b>25</b>	3.57, d (5.4)	66.7	3.59, d (5.3)	72.7	3.62, d (5.0)	66.2	3.62, d (4.8)	72.2	4.10, dd (10.4, 4.5)	68.1
<b>R<sub>1</sub></b>	<i>N</i> -CH <sub>3</sub>		<i>N</i> -CH <sub>3</sub>		<i>N</i> -CH <sub>3</sub>		<i>N</i> -CH <sub>3</sub>		<i>N</i> -CH <sub>3</sub>	
	2.68, s	31.8	2.92, s	40.3	2.66, s	31.8	2.91, s	39.7	2.97, s	41.1
<b>R<sub>2</sub></b>			<i>N</i> -CH <sub>3</sub>				<i>N</i> -CH <sub>3</sub>		<i>N</i> -CH <sub>3</sub>	
			2.92, s	41.9			2.91, s	42.2	2.97, s	41.1
<b>R<sub>3</sub></b>	Val		Val		iLeu		iLeu		Phe	
<b>1'</b>	2.13, m	30.2	2.45, m	27.2	1.88, m	36.8	2.16, m	33.9	3.12, dd (13.8, 10.6)	34.3
									3.40, m	
<b>2'</b>	0.95, d (6.7)	16.7	0.93, d (6.7)	15.1	1.02, m	25.1	0.77, m	26.3		134.1
					1.47, m		1.42, m			
<b>3'</b>	0.96, d (6.7)	17.5	0.97, d (6.7)	18.7	0.92, t (6.8)	10.3	0.93, t (6.9)	10.4	7.20, m	129.0
<b>4'</b>					0.96, d (6.8)	13.1	0.98, d (6.9)	11.5	7.30, m	128.5
<b>5'</b>									7.21, m	127.5
<b>6'</b>									7.30, m	128.5
<b>7'</b>									7.20, m	129.0

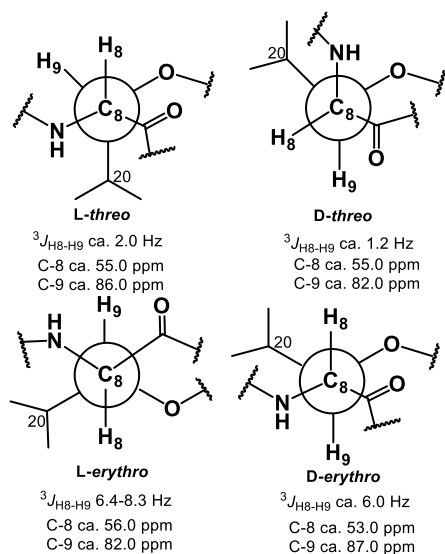
186

187

188 The stereochemistry of the cyclopeptide alkaloids **1** - **6**, was proposed from the <sup>1</sup>H NMR  
189 coupling constants, <sup>13</sup>C NMR data, and NOESY analysis and by determining the absolute  
190 configuration of the amino acids by chiral HPLC after acid hydrolysis. With this purpose,  
191 compounds **1** - **6** were hydrolyzed and their amino acids analyzed through the chiral HPLC. In  
192 cycloheisterin A-E and **6**, proline has the L configuration and *N*-methyl-valine, *N,N*-dimethyl-  
193 valine, *N*-methyl-isoleucine, *N,N*-dimethyl-isoleucine, and *N,N*-dimethyl phenylalanine in  
194 cycloheisterin A-E, respectively and *N,N*-dimethyl-leucine in **6** were in the L form. The <sup>13</sup>C-  
195 NMR chemical shift values of the  $\alpha$ -amino acid of the macrocycle (proline in all five alkaloids)  
196 and the terminal units (*N*-methyl-valine in **1**, *N,N*-dimethyl-valine in **2**, *N*-methyl-isoleucine in  
197 **3**, *N,N*-dimethyl-isoleucine in **4**, *N,N*-dimethyl-phenylalanine in **5**, and *N,N*-dimethyl-leucine  
198 in **6**) match well with those previously reported for similar compounds and was in agreement  
199 with the fact that the majority of plant cyclopeptides are composed of L-amino acids(El-Seedi  
200 et al. 2005, Kang et al. 2015, Maldaner et al. 2011, Medina et al. 2016, Suksamrarn et al. 2005,  
201 Tuenter et al. 2017).

202 The configuration of the  $\beta$ -hydroxyleucine was established based on the available NMR data.  
203 In the case of the *erythro* form,  $J_{\alpha,\beta}$  ca. 8.0 Hz, whereas for *threo* compounds  $J_{\alpha,\beta}$  ca. 2.0 Hz  
204 (Fig. 3) (Dias et al. 2007, Gournelis et al. 1997, Mostardeiro et al. 2013, Tuenter et al. 2016).  
205 The coupling constant of the doublet corresponding to H-9 ( $J_{\alpha,\beta}$ ) of compounds **1** – **5** ca. 8.3  
206 Hz, clearly indicative of an *erythro* configuration. <sup>13</sup>C NMR spectroscopy is used for the  
207 elucidation of the absolute configuration of the  $\beta$ -hydroxy amino acids. For both *L-threo* and *D-*  
208 *threo* series, the signal of the  $\alpha$  carbon appears at ca.  $\delta_C$  55.0, whereas for the  $\beta$  carbon, its signal  
209 appears at ca.  $\delta_C$  82.0 for the *D-threo* and ca.  $\delta_C$  86.0 for the *L-threo* (Fig. 3) (Mostardeiro et al.  
210 2013). For the *L-erythro* series, the signal of the  $\alpha$  carbon (C-8) appears at ca.  $\delta_C$  55.0, whereas  
211 for the *D-erythro* it appears at ca.  $\delta_C$  53.0. Important information is also observed for the  $\beta$   
212 carbon (C-9): in the *L-erythro* series, the signal appears at ca.  $\delta_C$  81.5, whereas for the *D-erythro*  
213 configuration it appears at ca.  $\delta_C$  87.0 (Abu-Zarga et al. 1995, Caro et al. 2012, Dongo et al.  
214 1989, Gournelis et al. 1997, Medina et al. 2016, Mostardeiro et al. 2013, Tuenter et al. 2016).  
215 These data show that the chemical shift of the  $\beta$  carbon is most indicative for the L and D forms  
216 of a  $\beta$ -hydroxy amino acids ( $\Delta\delta$  4 - 5 ppm) than  $\alpha$  carbon ( $\Delta\delta$  0 - 3 ppm). The chemical shift of  
217 C-9 in compounds **1** – **5** was around  $\delta_{C-9}$  83.3, clearly suggestive for the *L-erythro* form, whereas  
218 the chemical shift of C-8 was around  $\delta_C$  53.0. Furthermore, the *J* value of the <sup>1</sup>H NMR signal  
219 attributed to the methyl group at position C-22 was 6.7 Hz, indicative for a  
220 pseudoaxial/equatorial coupling, typical for *L-erythro-β*-hydroxyleucine (Abu-Zarga et al.

221 1995, Gournelis et al. 1997, Tuenter et al. 2016). In addition, the cross-peak observed in the  
 222 NOESY spectra of **1** - **5** between H-9 and H-20, H-9/H-21 and H-8/H-22 and the lack of the  
 223 NOESY interaction between H-9 and H-8, suggests the *L-erythro* configuration for the  $\beta$ -  
 224 hydroxyleucine moiety (Fig. 2). Furthermore, the NOESY effect observed between H-25 and  
 225 H-1' indicated that these protons are co-facially oriented.



227 Fig. 3. Representatives and approximates NMR data for *threo* and *erythro*  $\beta$ -hydroxyleucine in cyclopeptide  
 228 alkaloids.

229

### 230 3. Conclusion

231 In summary, six compounds were isolated from the crude alkaloid extract of *H. parvifolia*  
 232 leaves, among them five previously undescribed cyclopeptide alkaloids from the 4(14) type, 4  
 233 amino acid constituents outside and the 14-atoms of the macrocycle (inside). Their structures  
 234 were established by different spectroscopic methods including 1D- and 2D-NMR experiments  
 235 as well as HR-ESI-MS analysis. Compound **6** (anorldianine) that has a unique substructure  
 236 containing proline, was previously isolated from *Heisteria nitida* (El-Seedi et al. 1999).  
 237 Compounds **1-5** were derivatives of anorldianine and differed in only the terminal amino acid  
 238 which was *N*-methyl-valine in **1**, *N,N*-dimethyl-valine in **2**, *N*-methyl-isoleucine in **3**, *N,N*-  
 239 dimethyl-isoleucine in **4**, and *N,N*-dimethyl-phenylalanine in **5**. Cyclopeptide alkaloids have  
 240 only been reported from a few families of the plant kingdom, in fact, they seem to be quite rare  
 241 and present in small quantities. This kind of cyclopeptide alkaloids was isolated only in  
 242 *Canthium anorldianum* (Rubiaceae) and *Heisteria nitida* (Olacaceae). Further phytochemical  
 243 investigation on *Heisteria* species are needed to verify wether anorldianine derivative  
 244 cyclopeptide alkaloids could be considered as a taxonomic markers for the genus *Heisteria*. The

245 cytotoxic activity of compounds **1-6** against the chronic myeloid leukemia (K562) cell line was  
246 evaluated. Only compounds **2**, **4** and **6** exhibited an antiproliferative activity at the  
247 concentration 100  $\mu$ M with cell growth inhibition of 46%, 44%, and 43%, respectively, whereas  
248 compounds **1**, **3**, and **5** showed cell growth inhibition of 13%, 19%, and 36%, respectively at  
249 the same concentration.

## 250 **4. Experimental**

### 251 *4.1. General experimental procedures*

252 Optical rotations were measured on a Perkin Elmer model 341 polarimeter (589 nm, 20 °C). IR  
253 spectra were obtained on a Nicolet Avatar 320 FT-IR spectrometer with KBr disks. NMR  
254 spectra were acquired in CD<sub>3</sub>OD on Bruker Avance DRX III 500 instruments (<sup>1</sup>H at 500 MHz  
255 and <sup>13</sup>C at 125 MHz). Standard pulse sequences and parameters were used to obtain 1D- (<sup>1</sup>H  
256 and <sup>13</sup>C) and 2D- (COSY, ROESY, HSQC and HMBC) NMR spectra. HR-ESI-MS experiments  
257 were performed using a Micromass Q-TOF high-resolution mass spectrometer (Manchester,  
258 UK). Mass spectra were recorded in the positive-ion mode in the range  $m/z$  100–2000, with a  
259 mass resolution of 20000 and an acceleration voltage of 0.7 kV. Flash chromatography was  
260 conducted on a Grace Reveleris system equipped with dual UV and ELSD detection using  
261 Grace® cartridges (Silica gel or RP-18). A prepacked RP-C<sub>18</sub> column (Phenomenex 250 x 15  
262 mm, Luna 5  $\mu$ ) was used for semi-preparative HPLC. The eluting mobile phase consisted of  
263 H<sub>2</sub>O with TFA (0.0025%) and CH<sub>3</sub>CN with a flow rate of 5 mL/min and the chromatogram was  
264 monitored at 210, 250, 270, and 300 nm. TLC was performed on precoated silica gel 60 F<sub>254</sub>  
265 Merck and compounds were visualized by spraying the dried plates with Dragendorff's reagent.

### 266 *4.2. Plant material*

267 The leaves of *Heisteria parvifolia* Sm. were collected in Agboville forest in August 2016. They  
268 are identified by Pr. Akke Assi in the national center florestic of Félix Houphouët-Boigny  
269 University of Côte d'Ivoire (Ake assi 11049).

### 270 *4.3. Extraction and isolation*

271 The dried powdered leaves of *H. parvifolia* (1 kg) were wetted with 50% aq. NH<sub>4</sub>OH (500 mL),  
272 macerated overnight and then percolated with 15 L of EtOAc. The organic solvent was  
273 concentrated under reduced pressure. The crude extract (26 g) was suspended in 2 L of EtOAc  
274 and extracted with an aqueous 2% H<sub>2</sub>SO<sub>4</sub> solution (3 x 2 L). The acid phase was made alkaline  
275 with aqueous NH<sub>3</sub> and extracted with 3 x 2 L of CHCl<sub>3</sub>. The CHCl<sub>3</sub> solution was washed with  
276 H<sub>2</sub>O (2 L), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated *in vacuo* to give 500 mg of crude alkaloid extract  
277 (yield 0.05%). The crude alkaloid extract was subjected to silica gel flash chromatography

278 eluted with increasingly polar CHCl<sub>3</sub>/MeOH (100:00-95:05) for 25 min, to yield 26 fractions  
279 (F1-26). Fractions F6, F8, F10, F12, F14 and F17 were subjected separately to semipreparative  
280 HPLC RP-18 chromatography, by eluting with an isocratic gradient (28% CH<sub>3</sub>CN). Compound  
281 **4** (*t<sub>R</sub>* 13.2 min, 31 mg) was obtained from fractions F6 and F8, compound **5** (*t<sub>R</sub>* 14.9 min, 4 mg)  
282 from fraction F10, compound **6** (*t<sub>R</sub>* 10.6 min, 6 mg) from fraction F12, compounds **2** (*t<sub>R</sub>* 14.6  
283 min, 6 mg) and **3** (*t<sub>R</sub>* 17.3 min, 4 mg) from fraction F14, and compound **1** (*t<sub>R</sub>* 11.3 min, 5 mg)  
284 from fraction F17.

#### 285 4.3.1. Cycloheisterin A (**1**)

286 White amorphous powder;  $[\alpha]_D^{20} = -148$  (*c* 0.5; MeOH); UV (MeOH)  $\lambda_{\max}$  (abs.) 222 (1.66),  
287 274 (0.33); IR  $\nu_{\max}$  3395, 2972, 1682, 1508, 1205, 1133, 984, 720; <sup>1</sup>H and <sup>13</sup>C NMR, see Table  
288 1; HR-ESI-MS (positive ion mode) *m/z* 457.2807 [M + H]<sup>+</sup> (calcd for C<sub>25</sub>H<sub>37</sub>N<sub>4</sub>O<sub>4</sub>, 457.2815).

#### 289 4.3.2. Cycloheisterin B (**2**)

290 White amorphous powder;  $[\alpha]_D^{20} = -187$  (*c* 0.52; MeOH); UV (MeOH)  $\lambda_{\max}$  (abs.) 222 (0.10),  
291 282 (0.01); IR  $\nu_{\max}$  3439, 2969, 1681, 1508, 1204, 1136, 700; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1;  
292 HR-ESI-MS (positive ion mode) *m/z* 471.2979 [M + H]<sup>+</sup> (calcd for C<sub>26</sub>H<sub>39</sub>N<sub>4</sub>O<sub>4</sub>, 471.2971).

#### 293 4.3.3. Cycloheisterin C (**3**)

294 White amorphous powder;  $[\alpha]_D^{20} = -135$  (*c* 0.31; MeOH); UV (MeOH)  $\lambda_{\max}$  (abs.) 224 (1.38),  
295 276 (0.37); IR  $\nu_{\max}$  3388, 2965, 1686, 1506, 1206, 1133, 985, 719; <sup>1</sup>H and <sup>13</sup>C NMR, see Table  
296 1; HR-ESI-MS (positive ion mode) *m/z* 493.2785 [M + Na]<sup>+</sup> (calcd for C<sub>26</sub>H<sub>38</sub>N<sub>4</sub>O<sub>4</sub>Na,  
297 493.2791).

#### 298 4.3.4. Cycloheisterin D (**4**)

299 White amorphous powder;  $[\alpha]_D^{20} = -179$  (*c* 0.23; MeOH); UV (MeOH)  $\lambda_{\max}$  (abs.) 222 (3.21),  
300 280 (0.3); IR  $\nu_{\max}$  3395, 2972, 1682, 1508, 1205, 1133, 720; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; HR-  
301 ESI-MS (positive ion mode) *m/z* 485.3138 [M + H]<sup>+</sup> (calcd for C<sub>27</sub>H<sub>41</sub>N<sub>4</sub>O<sub>4</sub>, 485.3128).

#### 302 4.3.5. Cycloheisterin E (**5**)

303 White amorphous powder;  $[\alpha]_D^{20} = -91$  (*c* 0.41; MeOH); UV (MeOH)  $\lambda_{\max}$  (abs.) 222 (0.91),  
304 274 (0.5); IR  $\nu_{\max}$  3439, 2969, 1681, 1508, 1204, 1136, 700; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; HR-  
305 ESI-MS (positive ion mode) *m/z* 541.2799 [M + Na]<sup>+</sup> (calcd for C<sub>30</sub>H<sub>38</sub>N<sub>4</sub>O<sub>4</sub>Na, 541.2791).

#### 306 4.4. General procedure for determination of amino acid configurations.

307 The absolute configurations of amino acids were determined by chiral HPLC after acid  
308 hydrolysis according to literature ([Mostardeiro et al. 2013](#), [Siva et al. 1996](#), [Wang et al. 2017](#)).  
309 Briefly, each solution of **1-5** (0.5 mg) in 6 N HCl (0.4 mL) was heated at 110 °C for 24 h and  
310 then concentrated to dryness. The residue was dissolved in H<sub>2</sub>O (200 μL) to obtain the test

311 solution, 10  $\mu$ L of which was injected into chiral HPLC system with a Chiralpak IC column  
312 (250 mm  $\times$  4.6 mm I.D., 5  $\mu$ m) maintained at 35  $^{\circ}$ C and detected at 254 nm. : Isopropanol/*n*-  
313 hexane (90:10, v/v) containing 0.1% TFA was used as the mobile phase at a flow rate of 0.8  
314 mL/min.

### 315 **5. Cytotoxicity bioassay by MTS**

316 K562 cells (chronic myeloid leukemia) were trypsinized, harvested, and spread onto 96-well  
317 flat-bottom plates at a density of 1000 cells per well, and then incubated for 24 h in RPMI 1640  
318 Medium supplemented with 10% fetal bovine serum and antibiotics. After culture, the cells  
319 were treated with compounds **1-6** for 72 h. The cell cultures were then analyzed using 3-(4,5-  
320 dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner  
321 salt (MTS) according to the manufacturer's instructions (Promega Corporation, Charbonnières,  
322 France). Camptothecin was used as positive control. MTS is bio-reduced by cells into a colored  
323 formazan product. Absorbance was analyzed at a wavelength of 540 nm with a Multiskan Ex  
324 microplate absorbance reader (Thermo Scientific, Paris, France). Percentage of cell growth was  
325 calculated as 100%  $\times$  (absorbance of the treated cells) / (absorbance of the negative control  
326 cells). Control cells were treated with complete culture medium containing 0.2% DMSO. The  
327 values represent averages of three independent experiments.

### 328 **Supporting Information**

329 HR-ESI-MS and 1D- and 2D-NMR spectra of compounds **1-5**.

### 330 **Acknowledgements**

331 The authors are grateful to Conseil Regional Champagne Ardenne, Conseil General de la  
332 Marne, Ministry of Higher Education, Research and Innovation (France) (MESRI) and EU-  
333 programme FEDER to the PIAnET CPER project for financial support as well as the Ministry  
334 of Research of Côte d'Ivoire.

335

### 336 **References**

- 337 Abbiw, D., 1990. Useful Plants of Ghana: West African Uses of Wild and Cultivated Plants.  
338 London: Intermediate Technology Publications, Royal Botanic Gardens, Kew.
- 339 Abu-Zarga, M., Sabri, M., Al-Aboudi, A., 1995. New cyclopeptide alkaloids from *Ziziphus*  
340 *lotus*. *J. Nat. Prod.* 58, 504-511.
- 341 Burkill, H.M., 1997. The useful plants of West Tropical Africa. Edition 2, Vol. 4, families M-  
342 R, 284-285. Kew Royal Botanic Gardens.

343 Cairo-Valera, G., De Budowski, J., Delle-Monache, F., Marini-Bettolo, G.B., 1977. A new  
344 psychoactive drug : *Heisteria olivae* (Olacaceae). Atti Accad. Naz. Lincei, Cl. Sci. Fis. Mat.  
345 Nat. Rend. 62, 363-364.

346 Caro, M.S., de Oliveira, L.H., Ilha, V., Burrow, R.A., Dalcol, I.I., Morel, A.F., 2012. Absolute  
347 configuration of franganine. J. Nat. Prod. 75, 1220-1222.

348 Dias, G.C., Gressler, V., Hoenzel, S.C., Silva, U.F., Dalcol, I.I., Morel, A.F., 2007. Constituents  
349 of the roots of *Melochia chamaedrys*. Phytochemistry 68, 668-672.

350 Dirsch, V., Wiemann, W., Wagner, H., 1992. Anti-inflammatory activity of triterpene quinone-  
351 methides and proanthocyanidins from the stem bark of *Heisteria pallida* Engl. Pharm.  
352 Pharmacol. Lett., 2, 184-186.

353 Dirsch, V., Neszmélyi, A., Wagner, H., 1993. A trimeric propelargonidin from stem bark of  
354 *Heisteria pallida*. Phytochemistry 34, 291-293.

355 Dongo, E., Ayafor, J.F., Sondengam, B.L.; Connolly, J.D., 1989. A new peptide alkaloid from  
356 *Canthium anorldianum*. J. Nat. Prod. 52, 840-843.

357 El-Seedi, H.R., Gohil, S., Perera, P., Torssell, K.B.G., Bohlin, L., 1999. Cyclopeptide alkaloids  
358 from *Heisteria nitida*. Phytochemistry 52, 1739-1744.

359 El-Seedi, H.R., Larson, S., Backlund, A., 2005. Chemosystematic value of cyclopeptide  
360 alkaloids from *Heisteria nitida* (Olacaceae). Biochem. Syst. Ecol. 33, 831-839.

361 El-Seedi, H.R., Zahra, M.H., Göransson, U., Verpoorte, R., 2007. Cyclopeptide alkaloids.  
362 Phytochem. Rev. 6, 143-165.

363 Gournelis, D.C., Laskaris, G.G., Verpoorte, R., 1997. Cyclopeptides alkaloids. Nat. Prod. Rep.  
364 14, 75-82.

365 Joullie, M.M., Richard, D.J., 2004. Cyclopeptide alkaloids: chemistry and biology. Chem.  
366 Comm. 18, 2011-2015.

367 Kang, K.B., Ming, G., Kim, G.J., Ha, T.K., Choi, H, Oh, W.K., Sung, S.H., 2015. Jubanines F-  
368 J, cyclopeptide alkaloids from the roots of *Ziziphus jujuba*. Phytochemistry 119, 90-95.

369 Kraus, C.M., Neszmélyi, A., Holly, S., Wiedemann, B., Nenninger, A., Torssell, K.B., Bohlin,  
370 L., Wagner, H., 1998. New acetylenes isolated from the bark of *Heisteria acuminata*. J. Nat.  
371 Prod. 61, 422-427.

372 Kvist, L.P., Holm-Nielsen, L.B., 1987. Ethnobotanical aspects of lowland Ecuador. Opera  
373 Botanica, 92, 83-107.

374 Louppe, D., Oteng-Amoako, A.A., Brink, M., in Timbers, Plant Resources of Tropical Africa  
375 (Series), 7(1), pp: 385-386. Ed. Wageningen: PROTA Foundation: Backhuys, 2008.

376 Malaisse, F., N'Gasse, G., Lognay, G., 2004. *Heisteria parvifolia* (Olacaceae), an  
377 Underestimated shrub or small tree with Oil producing seeds. *Syst. Geogr. Plants.* 74, 7-25.  
378 Maldaner, G., Marangon, P., Ilha, V., Caro, M.S.P., Burrow, R.A., Dalcol, I.I., Morel, A.F.,  
379 2011. Cyclopeptide alkaloids from *Scutia buxifolia* Reiss. *Phytochemistry* 72, 804-809.  
380 Medina, R.P., Schuquel, I.T.A., Pomini, A.M., Silva, C.C., Oliveira, C.M.A., Kato, L.,  
381 Nakamura, C.V., Santin, S.M.O., 2016. Ixorine, a New Cyclopeptide Alkaloid from the  
382 Branches of *Ixora brevifolia*. *J. Braz. Chem. Soc.* 27, 753-758.  
383 Morel, A.F., Maldaner, G., Ilha, V., Missau, F., Silva, U.F., Dalcol, I.I., 2005. Cyclopeptide  
384 alkaloids from *Scutia buxifolia* Reiss and their antimicrobial activity. *Phytochemistry* 66,  
385 2571-2576.  
386 Morel, A.F., Maldaner, G., Ilha, V., 2009. Cyclopeptide Alkaloids from higher plants.  
387 Alkaloids. *Chem. Biol.* 67, 79-141.  
388 Mostardeiro, M.A., Ilha, V., dahmer, J., Caro, M.S.B., Dalcol, I.I., Da Silva, U.F., Morel, A.F.,  
389 2013. Cyclopeptide alkaloids: Stereochemistry and synthesis of the precursors of discarines  
390 C and D and myrianthine A. *J. Nat. Prod.* 76, 1343-1350.  
391 Russo, E.B., 1992. Headache treatments by native peoples of the Ecuadorian Amazon: a  
392 preliminary cross-disciplinary assessment. *J. Ethnopharmacol.* 36, 193-206.  
393 Schwing, K., Reyheller, C., Schaly, A., Kubik, S., Gerhards, M., 2011. Structural analysis of  
394 an isolated cyclic tetrapeptide and its monohydrate by combined IR/UV spectroscopy.  
395 *Chemphyschem.* 121981-1988.  
396 Silva, U.F., Cardoso, C.D., Zanatta, N., Icheln, D., Gehrcke, B., Morel, A.F., 1996.  
397 Determination of the stereochemistry of the *N,N*-dimethyl amino acid and the  $\alpha$ -amino acid  
398 residue of peptide alkaloids by chiral gas chromatography. *Phytochem. Anal.* 7, 20-23.  
399 Sugawara, F., Ishimoto, M., Le Van, N., Koshino, H., Uzawa, J., Yoshida, S., Kitamura, K.,  
400 1996. Insecticidal peptide from mung bean: a resistant factor against infestation with azuki  
401 bean weevil. *J. Agric. Food Chem.* 44, 3360-3364.  
402 Suh, D.Y., Kim, Y.C., Kang, Y.H., Han, B.H., 1997. Metabolic cleavage of frangufoline in  
403 rodents: *In Vitro* and *in Vivo* Study. *J. Nat. Prod.* 60, 265-269.  
404 Suksamrarn, S., Suwannapoch, N., Aunchai, N., Kuno, M., Ratananukul, P., Haritakun, R.,  
405 Jansakul, C., Ruchirawat, S., 2005. Ziziphine N, O, P and Q, new antiplasmodial  
406 cyclopeptide alkaloids from *Ziziphus oenoplia* var. *brunoniana*. *Tetrahedron* 61, 1175-1180.  
407 Tan, N.-H., Zhou, J., 2006. Plant cyclopeptides. *Chem. Rev.* 106, 840-895.  
408 Tuenter, E., Exarchou, V., Baldé, A., Cos, P., Maes, L., Apers, S., Pieters, L., 2016.  
409 Cyclopeptide alkaloids from *Hymenocardia acida*. *J. Nat. Prod.* 79, 1746-51.



- 410 Tuenter, E., Foubert, K., Staerk, D., Apers, S., Pieters, L., 2017. Isolation and structure  
411 elucidation of cyclopeptide alkaloids from *Ziziphus nummularia* and *Ziziphus spina-christi*  
412 by HPLC-DAD-MS and HPLC-PDA-(HRMS)-SPE-NMR. *Phytochemistry* 138, 163-169.
- 413 Wang, X., Wu, H., Luo, R., Xia, D., Jiang Z., Han, H., 2017. Separation and detection of free D- and  
414 L-amino acids in tea by off-line two-dimensional liquid chromatography. *Anal. Methods* 9, 6131-  
415 6138.