



## 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.

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

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

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

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

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

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

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

## Highlights

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

## ABSTRACT

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

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

## 1. Introduction

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

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

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

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

## 2. Results and discussion

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

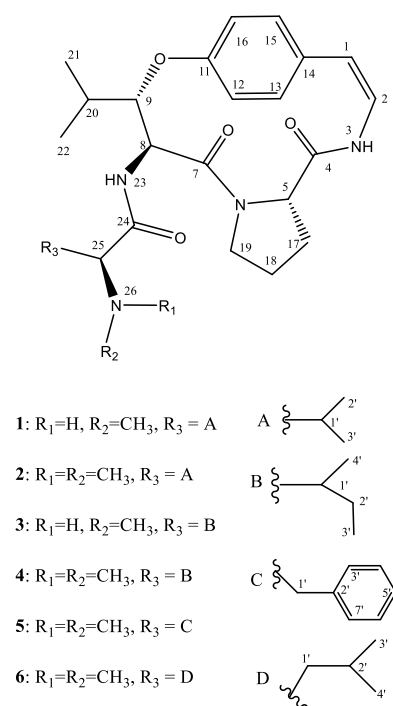


Fig. 1. Chemical structures of compounds **1-6**.

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

Compound **1** was obtained as a white powder. Analysis of **1** by high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) identified a pseudo-molecular ion  $[\text{M} + \text{H}]^+$  at  $m/z$  457.2807, corresponding to the molecular formula  $\text{C}_{25}\text{H}_{36}\text{N}_4\text{O}_4$  (calcd for  $\text{C}_{25}\text{H}_{37}\text{N}_4\text{O}_4$ , 457.2815), in combination with analysis of NMR data. The  $^{13}\text{C}$  NMR (Table 1) and HSQC spectra of **1** showed 25 carbon resonances for four methyls ( $\delta_{\text{C}}$  13.9, 16.7, 17.5, and 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 of which were olefinic carbons at  $\delta_{\text{C}}$  116.7 and 124.9 and four were aromatic carbons  $sp^2$  at  $\delta_{\text{C}}$  120.9, 121.9, 131.0, and 131.4), two quaternary aromatic carbons ( $\delta_{\text{C}}$  157.2 and 131.6), and three carbonyl carbons ( $\delta_{\text{C}}$  171.1, 167.5, and 165.2). The  $^1\text{H}$  NMR spectrum (Table 1) displayed 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 doublets, four aromatic protons, and several methine and methylene protons. The NMR data of compound **1** (Table 1) showed great similarity with the NMR data previously reported for anorldianine (compound **6**) possessing a 14-membered ring type comprising a *p*-oxygenated *Z*-styrylamine group (Dongo et al. 1989, El-Seedi et al. 1999, El-Seedi et al. 2005). The presence of the *p*-oxygenated *Z*-styrylamine group was indicated by two doublets at  $\delta_{\text{H}}$  6.53 and 6.65 (each 1H,  $J = 7.7$  Hz; H-1 and H-2, respectively) corresponding to the *Z*-double bond and four aromatic protons appearing as doublets of doublets with *J*-values typical for an *o,m*-coupling 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 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 ( $\delta_{\text{C}}$  131.6) in HMBC spectrum. In cyclopeptide alkaloids, the H-9 ( $\beta$ -H of the  $\beta$ -hydroxy-amino acid moiety) chemical shift value (between 5.00 and 5.50 ppm) is characteristic. In this case, a doublets of doublets was present at  $\delta_{\text{H}}$  4.92 ( $J = 8.3, 1.5$  Hz). In the COSY spectrum, two cross 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 H-20 ( $\delta_{\text{H}}$  2.13, sept,  $J = 6.9$  Hz). The proton signal of the CH-group in position 20 also showed 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 spectrum, correlations between C-9 ( $\delta_{\text{C}}$  83.4) and H-21 and H-22 were observed (Fig. 2). These data agreed with previously reported data for  $\beta$ -hydroxyleucine (anorldianine). The methine and the methylene protons of proline were observed in the  $^1\text{H}$  NMR spectrum: H-5 ( $\delta_{\text{H}}$  4.16, 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),

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 between H-5/H-17, H-17/H-18, and H-18/H-19. In the HMBC spectrum, the H-5 exhibited correlations with C-19 ( $\delta_{\text{C}}$  46.7) and with the carbonyl C-4 ( $\delta_{\text{C}}$  167.5) (Fig. 2). The methyl 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 Hz) and the methine proton (H-1') of this moiety appeared as septuplet at  $\delta_{\text{H}}$  2.13 ( $J$  = 6.9 Hz). The HMBC experiment showed correlations between C-24 ( $\delta$  165.2) and H-25 ( $\delta_{\text{H}}$  3.57, d,  $J$  = 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 combined use of 1D (<sup>1</sup>H and <sup>13</sup>C NMR) and 2D (COSY, HSQC, and HMBC) spectra allowed an unambiguous assignment of all protons and carbons of the amino acids units ( $\beta$ -hydroxyleucine, proline, *N*-methyl-valine residues) and the *p*-oxygenated *Z*-styrylamine group (Table 1). Moreover, the connectivity between the constitutive parts of the molecule were ascertained by the HMBC correlations between the carbonyl C-4/H-2, the carbonyl C-7/H-5, the carbonyl C-24/H-8, and C-11/H-9 (Fig. 2). Moreover, the NOE relationships H-8/H-25, H-8/H-12 and H-9/H-16 agreed with the  $\beta$ -hydroxyleucine connection with the *N*-methyl-valine and the *p*-oxygenated *Z*-styrylamine moieties whereas the NOE effects between H-8/H-19 agreed with the connection of  $\beta$ -hydroxyleucine with proline (Fig. 2). Compound **1** was named cycloheisterin A after its plant origin (Fig. 1).

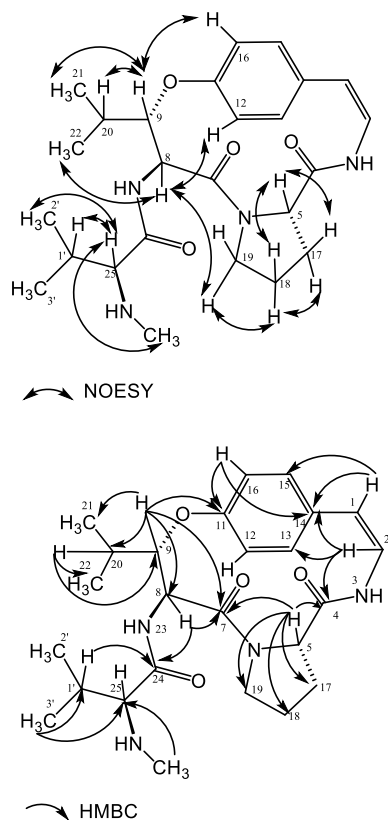


Fig. 2. Selected key HMBC and NOESY interactions for compound **1**.

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

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

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

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



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

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

	<b>1</b>		<b>2</b>		<b>3</b>		<b>4</b>		<b>5</b>	
	$\delta_{\text{H}}$ 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

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

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

1995, Gournelis et al. 1997, Tuentner et al. 2016). In addition, the cross-peak observed in the 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 NOESY interaction between H-9 and H-8, suggests the *L-erythro* configuration for the  $\beta$ -hydroxyleucine moiety (Fig. 2). Furthermore, the NOESY effect observed between H-25 and H-1' indicated that these protons are co-facially oriented.

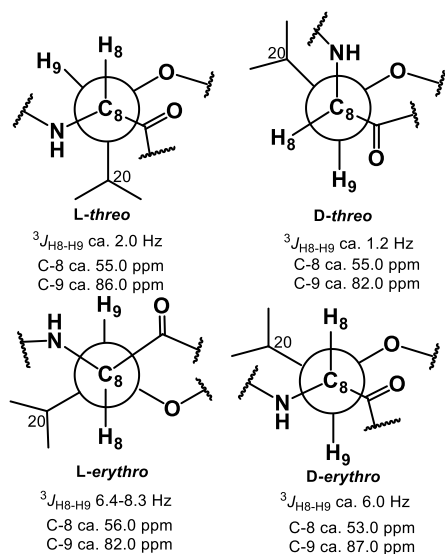


Fig. 3. Representatives and approximates NMR data for *threo* and *erythro*  $\beta$ -hydroxyleucine in cyclopeptide alkaloids.

### 3. Conclusion

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

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

## 4. Experimental

### 4.1. General experimental procedures

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

### 4.2. Plant material

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

### 4.3. Extraction and isolation

The dried powdered leaves of *H. parvifolia* (1 kg) were wetted with 50% aq. NH<sub>4</sub>OH (500 mL), macerated overnight and then percolated with 15 L of EtOAc. The organic solvent was concentrated under reduced pressure. The crude extract (26 g) was suspended in 2 L of EtOAc 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 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 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 (yield 0.05%). The crude alkaloid extract was subjected to silica gel flash chromatography

eluted with increasingly polar CHCl<sub>3</sub>/MeOH (100:00-95:05) for 25 min, to yield 26 fractions (F1-26). Fractions F6, F8, F10, F12, F14 and F17 were subjected separately to semipreparative HPLC RP-18 chromatography, by eluting with an isocratic gradient (28% CH<sub>3</sub>CN). Compound **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) 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 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) from fraction F17.

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

White amorphous powder;  $[\alpha]^{20}_D = -148$  (*c* 0.5; MeOH); UV (MeOH)  $\lambda_{\max}$  (abs.) 222 (1.66), 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 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).

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

White amorphous powder;  $[\alpha]^{20}_D = -187$  (*c* 0.52; MeOH); UV (MeOH)  $\lambda_{\max}$  (abs.) 222 (0.10), 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; 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).

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

White amorphous powder;  $[\alpha]^{20}_D = -135$  (*c* 0.31; MeOH); UV (MeOH)  $\lambda_{\max}$  (abs.) 224 (1.38), 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 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, 493.2791).

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

White amorphous powder;  $[\alpha]^{20}_D = -179$  (*c* 0.23; MeOH); UV (MeOH)  $\lambda_{\max}$  (abs.) 222 (3.21), 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-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).

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

White amorphous powder;  $[\alpha]^{20}_D = -91$  (*c* 0.41; MeOH); UV (MeOH)  $\lambda_{\max}$  (abs.) 222 (0.91), 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-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).

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

The absolute configurations of amino acids were determined by chiral HPLC after acid hydrolysis according to literature (Mostardeiro et al. 2013, Siva et al. 1996, Wang et al. 2017). 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 then concentrated to dryness. The residue was dissolved in H<sub>2</sub>O (200 µL) to obtain the test

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

## 5. Cytotoxicity bioassay by MTS

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

## Supporting Information

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

## Acknowledgements

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

## References

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