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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 medecine against numerous diseases in Côte d'Ivoire. Due to the shortcoming in scientifical 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 antiplasmodiale 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 dropps 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 administrated 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 $CHCl_3$/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.

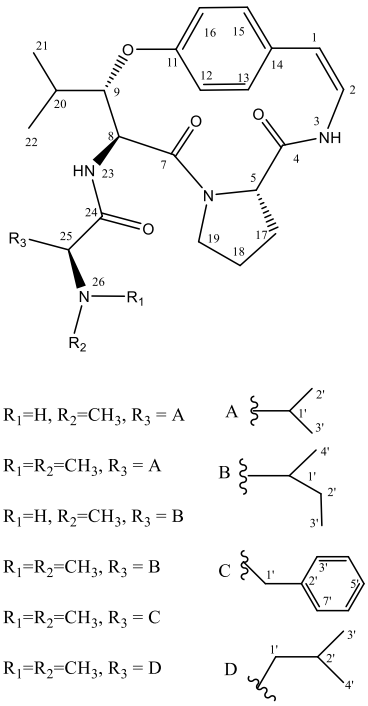

Fig. 1. Chemical structures of compounds **1**-**6**.

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 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 $[M + H]^+$ at *m/z* 457.2807, corresponding to the molecular formula $C_{25}H_{36}N_4O_4$ (calcd for $C_{25}H_{37}N_4O_4$, 457.2815), in combination with analysis of NMR data. The ^{13}C NMR (Table 1) and HSQC spectra of **1** showed 25 carbon resonances for four methyls (δ_C 13.9, 16.7, 17.5, and 19.5), one *N*-methyl (δ_C 31.8), three methylenes (δ_C 23.3, 28.2, and 46.7), twelve methines (two of which were olefinic carbons at δ_C 116.7 and 124.9 and four were aromatic carbons sp^2 at δ_C 120.9, 121.9, 131.0, and 131.4), two quaternary aromatic carbons (δ_C 157.2 and 131.6), and three carbonyl carbons (δ_C 171.1, 167.5, and 165.2). The ^{1}NMR spectrum (Table 1) displayed signals for two olefinic protons at δ_H 6.53 and 6.65, a singlet *N*-methyl (δ_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*-oxigenated *z*-styrylamine group (Dongo et al. 1989, El-Seedi et al. 1999, El-Seedi et al. 2005). The presence of the *p*-oxigenated *z*-styrylamine group was indicated by two doublets at δ_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 (δ_H 7.11, dd, J = 8.7, 2.4 Hz) showed correlation with C-1 (δ_C 116.7) and H-12 and H-16 (δ_H 7.27, dd, J = 8.7, 2.4 Hz) with C-14 (δ_C 131.6) in HMBC spectrum. In cyclopeptide alkaloids, the H-9 (*β*-H of the *β*-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 δ_H 4.92 (J = 8.3, 1.5 Hz). In the COSY spectrum, two cross peaks were observed for H-9 (δ_H 4.92), more specifically with H-8 (δ_H 5.01, d, J = 8.3 Hz) and H-20 (δ_H 2.13, sept, J = 6.9 Hz). The proton signal of the CH-group in position 20 also showed cross peaks to H_3-21 (δ_H 1.32, d, J = 6.7 Hz) and H_3-22 (δ_H 1.05, d, J = 6.7 Hz). In the HMBC spectrum, correlations between C-9 (δ_C 83.4) and H-21 and H-22 were observed (Fig. 2). These data agreed with previously reported data for *β*-hydroxyleucine (anorldianine). The methine and the methylene protons of proline were observed in the ^{1}H NMR spectrum: H-5 (δ_H 4.16, dd, J = 7.5, 1.9 Hz), H_2-17 (δ_H 1.65, m; 2.21, dd, J = 11.5-4.5 Hz), H_2-18 (δ_H 1.75, m; 1.95, m),

and H_2-19 (δ_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 (δ_C 46.7) and with the carbonyl C-4 (δ_C 167.5) (Fig. 2). The methyl doublets of *N*-methyl-valine (H-2' and H-3') appeared at δ_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 δ_H 2.13 (J = 6.9 Hz). The HMBC experiment showed correlations between C-24 (δ 165.2) and H-25 (δ_H 3.57, d, J = 5.4 Hz) and H-1', and between the *N*-methyl carbon (δ_C 31.8) and the H-25 for this moiety. The combined use of 1D (^{1}H and ^{13}C NMR) and 2D (COSY, HSQC, and HMBC) spectra allowed an unambiguous assignment of all protons and carbons of the amino acids units (*β*-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 *β*-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 *β*-hydroxyleucine with proline (Fig. 2). Compound **1** was named cycloheisterin A after its plant origin (Fig. 1).

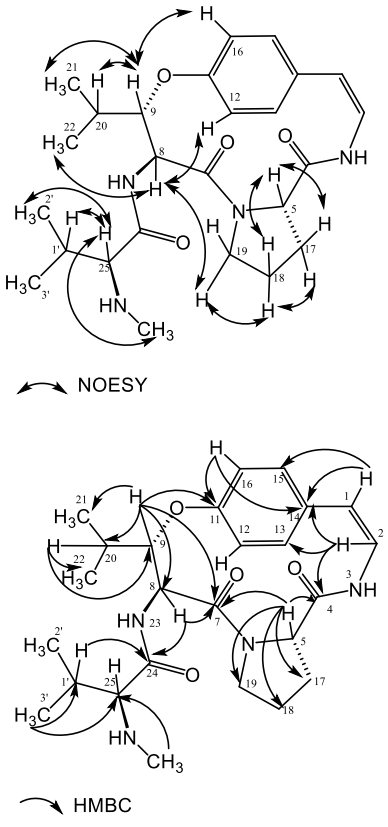

Fig. 2. Selected key HMBC and NOESY interactions for compound **1**.

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 (*β*-hydroxyleucine, proline, and valine residues) and the *p*-oxygenated *z*-styrylamine group (Table 1). The HMBC cross-peaks of the methyl signals at δ_H 2.92 (6H, s) to C-25 (δ_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 ($\delta_{H\text{-}8}$ 5.01) to C-24 of isoleucine residue ($\delta_{C\text{-}24}$ 165.3) and H-25 ($\delta_{H\text{-}25}$ 3.62) to C-24 and the *N*-methyl carbon (δ_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 (*β*-hydroxyleucine, proline and isoleucine residues) and the *p*-oxygenated *z*-styrylamine group (Table 1). The HMBC cross-peaks of the methyl signals at δ_H 2.91 (6H, s) to C-25 (δ_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 (*β*-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 [δ_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 δ_H 2.97 (6H, s) and C-25 (δ_C 68.1) of the phenylalanine residue. The HMBC correlation between H-8 (δ_H 4.82) of the β-hydroxyleucine and the C-24 (δ_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}**C** NMR spectroscopic data for compounds **1-5** (500 MHz, CD_3OD).

	1		**2**		**3**		**4**		**5**	
	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C
1	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
2	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
4	-	167.5	-	167.5	-	167.5	-	167.4	-	167.9
5	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
7	-	171.1	-	171.0	-	171.1	-	171.0	-	170.7
8	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
9	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
11	-	157.2	-	157.2	-	157.2	-	157.2	-	157.1
12	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
13	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
14	-	131.6	-	131.8	-	131.6	-	131.6	-	131.3
15	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
16	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
17	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)	
18	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	
19	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	
20	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
21	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
22	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
24	-	165.2	-	164.3	-	165.3	-	164.4	-	165.0
25	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
***R*$_1$**	*N*-CH_3		*N*-CH_3		*N*-CH_3		*N*-CH_3		*N*-CH_3	
	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
***R*$_2$**			*N*-CH_3				*N*-CH_3		*N*-CH_3	
			2.92, s	41.9			2.91, s	42.2	2.97, s	41.1
***R*$_3$**	Val		Val		iLeu		iLeu		Phe	
1'	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	
2'	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			
3'	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
4'					0.96, d (6.8)	13.1	0.98, d (6.9)	11.5	7.30, m	128.5
5'									7.21, m	127.5
6'									7.30, m	128.5
7'									7.20, m	129.0

The stereochemistry of the cyclopeptide alkaloids **1** - **6**, was proposed from the ^{1}H NMR coupling constants, ^{13}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}C-NMR chemical shift values of the *α*-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, Tuenter et al. 2017).

The configuration of the *β*-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, Tuenter 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}C NMR spectroscopy is used for the elucidation of the absolute configuration of the *β*-hydroxy amino acids. For both L-*threo* and D-*threo* series, the signal of the *α* carbon appears at ca. δ_C 55.0, wheras for the *β* carbon, its signal appears at ca. δ_C 82.0 for the D-*threo* and ca. δ_C 86.0 for the L-*threo* (Fig. 3) (Mostardeiro et al. 2013). For the L-*erythro* series, the signal of the *α* carbon (C-8) appears at ca. δ_C 55.0, wheras for the D-*erythro* it appears at ca. δ_C 53.0. Important information is also observed for the *β* carbon (C-9): in the L-*erythro* series, the signal appears at ca. δ_C 81.5, whereas for the D-*erythro* configuration it appears at ca. δ_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, Tuenter et al. 2016). These data show that the chemical shift of the *β* carbon is most indicative for the L and D forms of a *β*-hydroxy amino acids (Δ_δ 4 - 5 ppm) than *α* carbon (Δ_δ 0 - 3 ppm). The chemical shift of C-9 in compounds **1** – **5** was around $\delta_{C\text{-}9}$ 83.3, clearly suggestive for the L-*erythro* form, whereas the chemical shift of C-8 was around δ_C 53.0. Furthermore, the *J* value of the ^{1}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*-*β*-hydroxyleucine (Abu-Zarga et al.

1995, Gournelis et al. 1997, Tuenter 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 *β*-hydroxyleucine moiety (Fig. 2). Furthermore, the NOESY effect observed between H-25 and H-1' indicated that these protons are co-facially oriented.

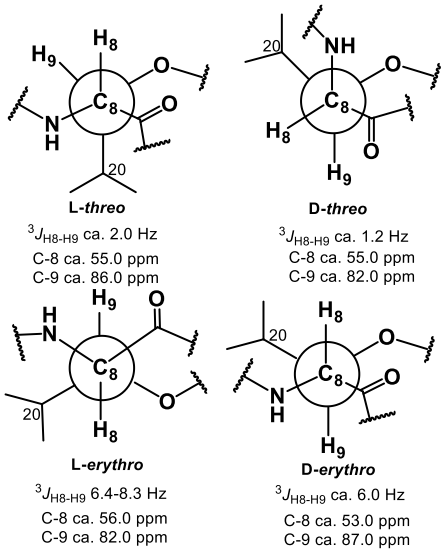

Fig. 3. Representatives and approximates NMR data for *threo* and *erythro* *β*-hydroxyleucine in cyclopeptide alkaloids.

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 wether 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 µM 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_3OD on Bruker Avance DRX III 500 instruments (^1H at 500 MHz and ^{13}C at 125 MHz). Standard pulse sequences and parameters were used to obtain 1D- (^1H and ^{13}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_{18} column (Phenomenex 250 x 15 mm, Luna 5 µ) was used for semi-preparative HPLC. The eluting mobile phase consisted of H_2O with TFA (0.0025%) and CH_3CN 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_{254} 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_4OH (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_2SO_4 solution (3 x 2 L). The acid phase was made alkaline with aqueous NH_3 and extracted with 3× 2 L of $CHCl_3$. The $CHCl_3$ solution was washed with H_2O (2 L), dried (Na_2SO_4) 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 $CHCl_3$/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_3CN). Compound **4** (t_R 13.2 min, 31 mg) was obtained from fractions F6 and F8, compound **5** (t_R 14.9 min, 4 mg) from fraction F10, compound **6** (t_R 10.6 min, 6 mg) from fraction F12, compounds **2** (t_R 14.6 min, 6 mg) and **3** (t_R 17.3 min, 4 mg) from fraction F14, and compound **1** (t_R 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) λ_{max} (abs.) 222 (1.66), 274 (0.33); IR ν_{max} 3395, 2972, 1682, 1508, 1205, 1133, 984, 720; ^1H and ^{13}C NMR, see Table 1; HR-ESI-MS (positive ion mode) *m/z* 457.2807 $[M + H]^+$ (calcd for $C_{25}H_{37}N_4O_4$, 457.2815).

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

White amorphous powder; $[\alpha]^{20}_D$ = -187 (*c* 0.52; MeOH); UV (MeOH) λ_{max} (abs.) 222 (0.10), 282 (0.01); IR ν_{max} 3439, 2969, 1681, 1508, 1204, 1136, 700; ^1H and ^{13}C NMR, see Table 1; HR-ESI-MS (positive ion mode) *m/z* 471.2979 $[M + H]^+$ (calcd for $C_{26}H_{39}N_4O_4$, 471.2971).

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

White amorphous powder; $[\alpha]^{20}_D$ = -135 (*c* 0.31; MeOH); UV (MeOH) λ_{max} (abs.) 224 (1.38), 276 (0.37); IR ν_{max} 3388, 2965, 1686, 1506, 1206, 1133, 985, 719; ^1H and ^{13}C NMR, see Table 1; HR-ESI-MS (positive ion mode) *m/z* 493.2785 $[M + Na]^+$ (calcd for $C_{26}H_{38}N_4O_4Na$, 493.2791).

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

White amorphous powder; $[\alpha]^{20}_D$ = -179 (*c* 0.23; MeOH); UV (MeOH) λ_{max} (abs.) 222 (3.21), 280 (0.3); IR ν_{max} 3395, 2972, 1682, 1508, 1205, 1133, 720; ^1H and ^{13}C NMR, see Table 1; HR-ESI-MS (positive ion mode) *m/z* 485.3138 $[M + H]^+$ (calcd for $C_{27}H_{41}N_4O_4$, 485.3128).

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

White amorphous powder; $[\alpha]^{20}_D$ = -91 (*c* 0.41; MeOH); UV (MeOH) λ_{max} (abs.) 222 (0.91), 274 (0.5); IR ν_{max} 3439, 2969, 1681, 1508, 1204, 1136, 700; ^1H and ^{13}C NMR, see Table 1; HR-ESI-MS (positive ion mode) *m/z* 541.2799 $[M + Na]^+$ (calcd for $C_{30}H_{38}N_4O_4Na$, 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_2O (200 μL) to obtain the test

311 solution, 10 μL of which was injected into chiral HPLC system with a Chiralpak IC column
312 (250 mm × 4.6 mm I.D., 5 μm) maintained at 35 °C and detected at 254 nm. : Isopropanol/*n*-
313 hexane (90:10, v/v) containig 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 bioreduced 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% × (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**.

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