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Activity-guided isolation of antiplasmodial dihydrochalcones and flavanones from *Piper hostmannianum* var. *berbicense*

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Abstract

The bioassay-guided purification of an *n*-hexane extract from the leaves of *Piper hostmannianum* var. *berbicense* led to the isolation of four monoterpene or prenyl-substituted dihydrochalcones (**1a**, **1b**, **2**, **3**) as well as the known compounds 2′,6′-dihydroxy-4′-methoxydihydrochalcone (**4**), linderatone (**5**), strobopinin (**6**), adunctin E (**7**) and (−)-methyllinderatin (**8**). Their structures were established on the basis of NMR and X-ray analysis. (−)-Methyllinderatin, linderatone and 2′,6′-dihydroxy-4′-methoxydihydrochalcone exhibited the most potent antiplasmodial activity with IC_{50} values of 5.64, 10.33 and 12.69 μM, respectively against both chloroquine-sensitive and resistant strains of *Plasmodium falciparum* (F32, FcB1). The activity of (−)-methyllinderatin was confirmed *in vivo* against *Plasmodium vinckei petteri* in mice (80% of reduction of parasitemia) at a dose of 20 mg/kg/day.

Keywords: *Piper hostmannianum* var. *berbicense*; Piperaceae; Dihydrochalcones; Flavanones; Antiplasmodial activity; *Plasmodium falciparum*

1. Introduction

Due to the rising prevalence of *Plasmodium falciparum* resistance to chloroquine and other antimalarial drugs, the treatment of malaria is becoming increasingly difficult (Hyde, 2002; Winstanley et al., 2002). There is an urgent need to discover new drugs that are safe and effective for the prophylaxis and treatment of malaria (Biagini et al., 2003). Previous findings of antimalarial agents such as quinine and artemisinin from medicinal plants also encouraged the possibility of finding new antimalarial drugs from plant source (Schwikkard and van Heerden, 2002). In part of our research aiming to uncover antimalarial agents from the biodiversity of French Guiana, the *n*-hexane extract of leaves of *Piper hostmannianum* var. *berbicense* (Miq.) C. DC. (Piperaceae) exhibited interesting activity against *Plasmodium falciparum* ($IC_{50} = 8$ μg/ml). The phytochemical investigation of the genus *Piper*, widely distributed in the tropical and subtropical regions of the world, has reported the isolation of several classes of antiprotozoal compounds such as alkaloids (Rukachaisirikul et al., 2002, 2004), lignans (Lee and Ley, 2003; Ma et al., 1991), chalcones and dihydrochalcones (Jenett-Siems et al., 1999). From a phytochemical point of view, this plant species is known to contain chromenes, flavanones and benzoic acid derivatives evaluated for their antifungic activity (Diaz et al., 1987; Lago et al., 2004). In the present investigation, an activity bioassay-guided fractionation of *n*-hexane extract by a variety of chromatographic methods has led to the isolation of four new dihydrochalcones (**1a, 1b, 2, 3**) as well as the known compounds 2′,6′-dihydroxy-4′-methoxydihydrochalcone (**4**) linderatone (**5**), strobopinin (**6**), adunctin E (**7**) and (−)-methyllinderatin (**8**)

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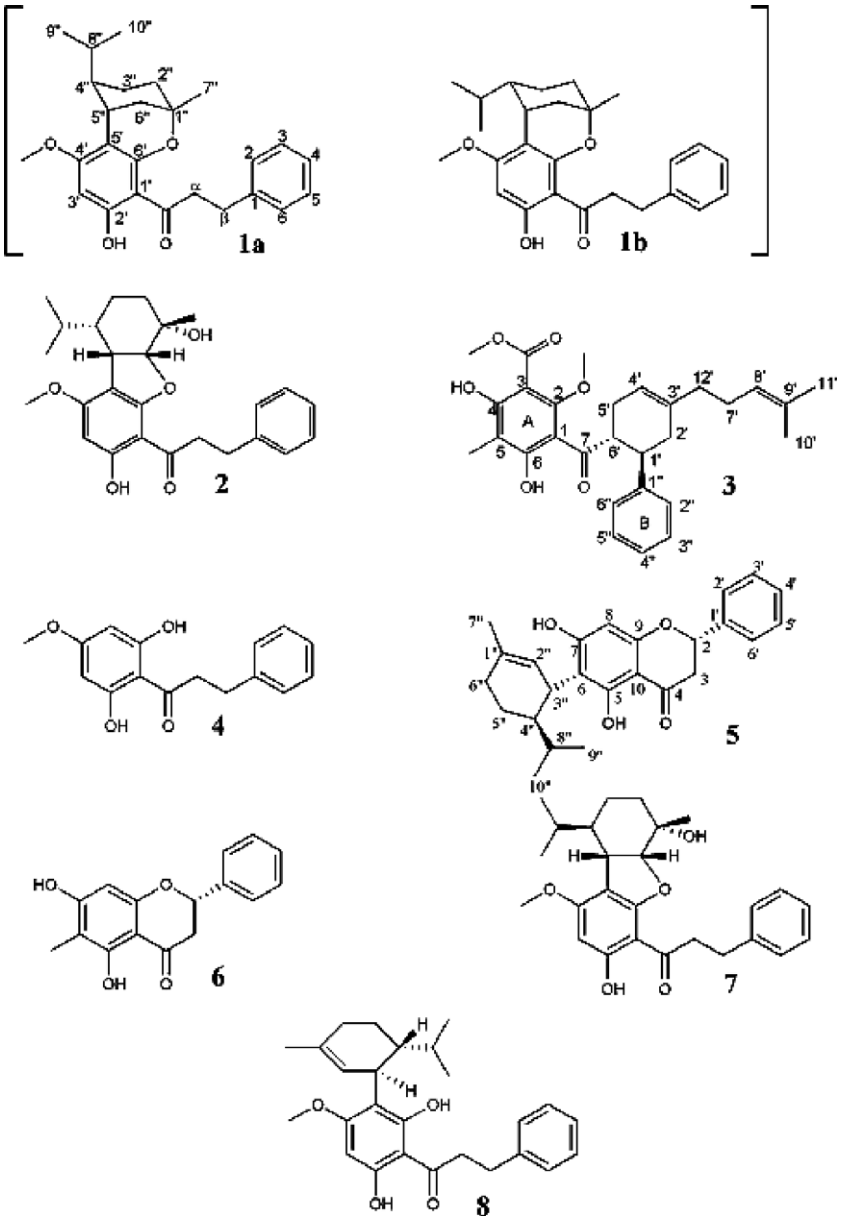

Fig. 1. Dihydrochalcones and flavanones isolated from the leaves of *P. hostmannianum* var. *berbicense*.

(see Fig. 1). The structure of **1** has been confirmed by means of X-ray crystallography. The *in vitro* activity of the isolated compounds was assessed against both chloroquine-resistant (FcB1) and chloroquine-sensitive (F32) strains of *Plasmodium falciparum* and MCF7 cells were used to evaluate the cytotoxicity. In addition, the major dihydrochalcone, (−)-methyllinderatin (**8**), was evaluated for *in vivo* antimalarial activity against *Plasmodium vinckei petteri* in mice.

2. Results and discussion

Compound **1** obtained as yellow prisms had the molecular formula $C_{26}H_{32}O_4$, determined by ESI-Q-TOF-HRMS at *m*/*z* 407.2242 $[M-H]^-$ (calcd. 407.2222). Its IR spectrum contained absorption bands due to hydrogen-bonded OH (3400 cm^{-1}), conjugated carbonyl (1616 cm^{-1}) and aryl (1585 cm^{-1}) moieties. Although the product appeared to be homogeneous by TLC, structurally related examination of the ^1H and ^{13}C NMR spectra indicated that it was still a mixture as there was slight doubling up of certain signals. Indeed, the ^{13}C NMR spectrum of **1** displayed a set of major peaks accompanied by corresponding less intense peaks in a ratio of approximately 55:45. However, various HPLC separations of compound **1** failed to resolve it, suggesting that the latter could be an inseparable mixture of two structurally close isomers. Structure elucidation was thus primarily based on the major peaks in its ^1H and ^{13}C NMR spectra. In the ^1H NMR spectrum of **1** (Table 1), signals characteristic of a 2′,6′-dihydroxy-4′-methoxy-dihydrochalcone derivative were clearly observed at δ_H

Table 1
^{1}H NMR spectral data for compounds **1–3** (500 MHz, in $CDCl_3$)[a]

H	**1a** (major dia)	**1b** (minor dia)	**2**	H	**3**
Dihydrochalcone moiety					
H-2 to H-6	7.21–7.29 *m*	7.21–7.29 *m*	7.21–7.31 *m*	1′	3.11 *ddd* (10.6, 10.6, 6.4)
H-α	3.49 *m*	3.49 *m*	3.41 *m*	2′	2.25[a]
H-β	3.06 *t* (8.0)	3.06 *t* (8.0)	3.07 *t* (7.9)	4′	5.55 *br s*
H-3′	6.02 *s*	6.01 *s*	6.09 *s*	5′	2.49 [a]
OH-2′	13.90 *s*	13.90	13.26 *s*	6′	4.21 *ddd* (10.2, 10.2, 5.4)
OCH_3-4′	3.83 *s*	3.80 *s*	3.84 *s*	$7'_a$	2.12 [a]
Monoterpene moiety				$7'_b$	2.03 [a]
H_a-2″	1.74 *m*	1.99 *m*	1.79 *m*	8′	5.13 *t* (6.8)
H_b-2″	1.61 *m*	1.53 *m*	1.68 *m*	10′	1.64 *s*
H_a-3″	1.53 *m*	1.63 *m*	1.21 *m*	11′	1.73 *s*
H_b-3″	–	–	1.67 *m*	12′	2.05, 2.13
H-4″	1.21 *m*	1.24 *m*	1.19 *m*	2″/6″	7.12
H-5″	3.39 *m*	3.49 *m*	3.07 t (5.7)	3″/5″	7.12
H_a-6″	1.91 *dd* (13.4, 3.4)	1.81 *m*		4″	7.06
H_b-6″	1.51 *dd* (13.4, 5.3)	1.73 *m*	4.54 *dd* (5.7, 1.2)	OH-6	12.23 *s*
CH_3-7″	1.36 *s*	1.37 *s*	1.41 *s*	OH-4	12.06 *s*
H-8″	1.78 *m*	1.22 *m*	1.88 *m*	OCH_3-2	3.79 *s*
CH_3-9″	1.08 *d* (6.8)	1.09 *d* (6.6)	0.90 *d* (7.0)	CH_3-5	1.90 *s*
CH_3-10″	0.97 *d* (6.8)	0.75 *d* (6.6)	0.86 *d* (7.0)	C=OOCH_3-3	4.03 *s*

[a] Multiplicity patterns were unclear due to signal overlapping.

13.90 (OH chelated), 7.21–7.29 (5H, *m*), δ_H 3.06 (2H, *t*), δ_H 3.49 (2H, *m*), δ_H 6.02 (1H, *s*) and δ_H 3.83 (3H, *s*) (Burke and Nair, 1986) supported by λ_{max} in the UV spectrum at 234 nm and 292 nm. The ^{13}C NMR spectrum confirmed the signals of the dihydrochalcone and contained ten additional resonances with three methyls at δ_C 29.2 (C-7″), δ_C 20.9 (C-9″), and δ_C 22.1 (C-10″), three methylene carbons (δ_C 30.1 (C-6″), δ_C 20.5 (C-3″), δ_C 35.3 (C-2″)), three methine carbons (δ_C 44.0 (C-4″), δ_C 27.2 (C-5″), δ_C 26.3 (C-8″)) and a quaternary carbon at δ_C 77.9 (C-1″), bearing an O-atom. The latter, associated with the molecular formula, suggested that the dihydrochalcone was substituted by a saturated cyclic monoterpene moiety (Tables 1 and 2). Indeed, the ^{1}H NMR spectrum showed signals characteristic of an isopropyl moiety (δ_H 1.78, 1.08, 0.97 (H-8″(Me)$_2$)) and a methyl group (δ_H 1.36 (H-7′)). This spectroscopic data (Tables 1 and 2) was quite similar to a *p*-menthane unit (Senda and Imaizumi, 1975). The position of the *p*-menthane unit on the dihydrochalcone moiety was established with the combined results of COSY, HSQC and HMBC experiments. The chemical shift of H-5″ at δ_H 3.39 was indicative of a CH group adjacent to an aryl moiety as seen in methyllinderatin (Ichino, 1989). This hypothesis was supported by HMBC correlations between H-5″ (δ_H 3.39) and C-5′ (δ_C 106.8) denoting that *p*-menthane was C-linked to the dihydrochalcone core between C-5″ and C-5′. In the HMBC spectrum (see correlations in Fig. 2), protons of the methyl group (δ_H 1.36) displayed correlations with the deshielded quaternary carbon at δ_C 77.9 (C-1″) and the aromatic carbon C-6′ (δ_C 158.9) of the dihydrochalcone which indicated that the *p*-menthane unit was O-linked to the dihydrochalcone unit between C-1″ and (O-(C-6′)) thus forming a six-membered ring between the *p*-menthane unit and the dihydrochalcone core. The proposed structures **1a** and **1b** (Fig. 1) with their relative configuration were eventually confirmed by a single-crystal X-ray analysis. The structure showed that the monoterpene unit adopted a chair conformation in which the methyl group at C-7″ appeared to be in equatorial position in

Table 2
^{13}C NMR data of compounds **1–3** (125 MHz in $CDCl_3$)

C	**1a** (major dia)	**1b** (minor dia)	**2**	C	**3**
Dihydrochalcone moiety					
1	141.8	141.8	141.2	1	111.2
2/6	128.3	128.3	128.2	2	163.8
3/5	128.4	128.4	128.6	3	99.4
4	125.8	125.9	126.2	4	164.8
α	45.5	45.5	44.2	5	108.6
β	30.7	30.7	30.6	6	164.2
C=O	204.9	204.8	203.4	7	210.8
1′	104.7	104.9	102.7	1′	45.1
2′	165.4	165.3	165.6	2′	37.3
3′	91.2	91.0	92.9	3′	137.4
4′	162.3	163.2	161.3	4′	118.9
5′	106.8	103.5	112.9	5′	30.2
6′	158.9	159.3	161.7	6′	51.5
CH_3O	55.7	55.1	55.5	7′	37.4
Monoterpene moiety				8′	124.1
1″	77.9	76.7	81.9	9′	131.7
2″	35.3	40.2	30.3	10′	17.7
3″	20.5	22.5	19.4	11′	25.8
4″	44.0	49.9	46.8	12′	26.4
5″	27.2	28.4	41.1	1″	143.8
6″	30.1	36.9	89.2	2″/6″	127.5
7″	29.2	28.7	21.2	3″/5″	128.1
8″	26.3	29.6	26.9	4″	126.2
9″	20.9	22.4	15.3	OCH_3-2	65.2
10″	22.1	20.4	21.8	CH_3-5	7.3
				C=OOCH_3	170.9
				C=OOCH_3	52.8

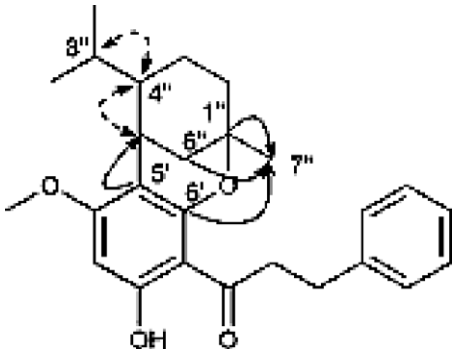

Fig. 2. Selected COSY (dotted line) and HMBC (solid line) correlations for **1a**.

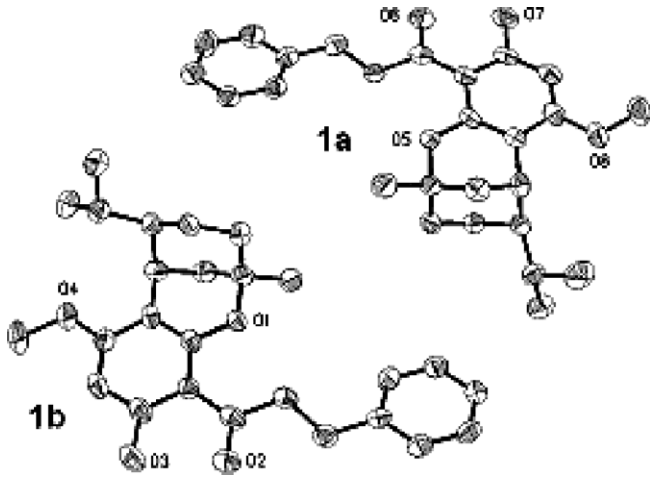

Fig. 3. ORTEP drawing showing the oxygen atom and solid-state conformation of **1a** and **1b**.

the two isomers. On the other hand, the position of the isopropyl group appeared to be in axial position in the major isomer **1a** and in equatorial position in the minor isomer **1b**. The NOESY spectrum confirmed this result by displaying interactions between H-5″ (δ_H 3.39) and H-4″ (δ_H 1.21) in the major isomer. As shown in Fig. 3, the two diastereoisomers which co-crystallized, were perfectly ordered and packed around an approximate inversion center. Although the absolute configuration could not be determined, it was arbitrarily assigned (Fig. 3). Therefore the structures of these two new monoterpene-substituted dihydrochalcones were elucidated as 2′-hydroxy-4′-methoxy-5′,6′-*O*-(4β-isopropyl-1β-methyl-cyclohexane-1-*O*-5-yl)dihydrochalcone (**1a**) and 2′-hydroxy-4′-methoxy-5′,6′-*O*-(4α-isopropyl-1β-methyl-cyclohexane-1-*O*,5-yl) dihydrochalcone (**1b**) and the trivial names hostmanin A and hostmanin B were proposed. Compound **2** obtained as a white amorphous powder was assigned the molecular formula $C_{26}H_{32}O_5$ from its ESI-Q-TOF-HRMS (*m*/*z* 425.2286 $[M+H]^+$ (calcd. 425.2328)) and suggested that **2** differed from **1** by an additional oxygen atom. All the spectral data of compound **2** (Tables 1 and 2) indicated it to be also a 5′-monoterpene-substituted 2′-6′-dihydroxy-4′-methoxydihydrochalcone derivative. Concerning this monoterpene moiety, the ^{13}C NMR spectrum displayed two methylene carbons (δ_C 30.3 (C-2″), 19.4 (C-3″)) and three methine carbons (δ_C 46.8 (C-4″), 41.1 (C-5″), 89.2 (C-6″)) instead of three methylene carbons (δ_C 35.3 (C-2″), 20.5 (C-3″), 30.1 (C-6″)) and two methine carbons (δ_C 44.0 (C-4″), 27.2 (C-5″)), for compound **1** as shown in Table 2. Thus the difference between **1** and **2** referred to the substitution of the saturated cyclic monoterpene moiety and the presence of an additional alcohol function in **2**. The low-field signal of the C-1″ (δ_C 81.9) suggested that this quaternary carbon bore the additional alcohol function. Thus the *p*-menthane unit might be linked to the dihydrochalcone core as in compound **1** with an hydroxyl group in C-6″. Nevertheless, this hypothesis was rejected. Indeed, in the COSY spectrum, correlation cross-peak was seen between H-5″ (δ_H 3.07) and H-6″ (δ_H 4.54) with a coupling constant of $J(H_{5''}, H_{6''}) = 5.7$ Hz. The latter consideration and the presence of the deshielded signal for H-6″ at δ_H 4.54 supported by HMBC correlation between H-5″ (δ_H 3.07) and C-6′ (δ_C 161.7) revealed that the second connectivity of the *p*-menthane unit was located at C-6″. Thus the *p*-menthane unit and the dihydrochalcone core formed a five-membered ring as in agreement with the spectral data of adunctin E previously isolated from *Piper aduncum* (Orjala et al., 1993). Nevertheless the 2D NOESY experiment revealed NOE interactions between H-4″ (δ_H 1.19)/H-5″ (δ_H 3.07), H-5″ (δ_H 3.07)/H-6″ (δ_H 4.54), and H-6″ (δ_H 4.54)/H-7″ (δ_H 1.41), thus establishing the relative configuration at (C-4″)/(C-5″) and (C-1″)/(C-6″), the isopropyl and methyl group (C-7″) being trans to each other. The structure of **2** was established as a diastereoisomer of adunctin E as (1″*R*,4″*R*,5″*S*,6″*S*)-2′-hydroxy-4′-methoxy-5′, 6′-*O*-(4-isopropyl-1-methyl-cyclohexan-1-ol-5, 6-*O*-yl) dihydrochalcone and named hostmanin C. Compound **3** was isolated as an optically inactive yellow oil. The molecular formula $C_{29}H_{34}O_6$ was determined by ESI-Q-TOF-HRMS at *m*/*z* 479.2367 $[M+H]^+$ (calcd. 479.2434). The infrared spectrum contained a broad band at 3500 cm^{-1} due to hydrogen-bonded OH, conjugated ester carbonyl (1700 cm^{-1}), conjugated carbonyl (1616 cm^{-1}) and aromatic ring (1550 cm^{-1}). The ^1H NMR spectrum of compound **3** exhibited signals assignable to a dimethylallyl group at δ_H 1.64 and δ_H 1.73, one aromatic methyl at δ_H 1.90, an aromatic methoxy group at δ_H 3.79, an aryl methyl ester at δ_H 4.03, two olefinic signals at δ_H 5.13 and δ_H 5.55, a monosubstituted phenyl group at δ_H 7.06–7.12 and two hydrogen-bonded OH at δ_H 12.06 and δ_H 12.23. The ^{13}C NMR spectrum further disclosed the presence of four methylene carbons (δ_C 37.4, 26.4, 37.3, 30.2), two methine carbons (δ_C 45.1 and 51.5) and six fully substituted sp^2 carbons attesting the presence of a second aromatic ring. This data led us to think that **3** should be a prenylated dihydrochalcone in which the ring A was totally substituted. This was supported by the comparison with the ^1H and ^{13}C NMR data of the known geranylated dihydrochalcone (±)-nicolaiodesin C (Gu et al., 2002) which essentially displayed

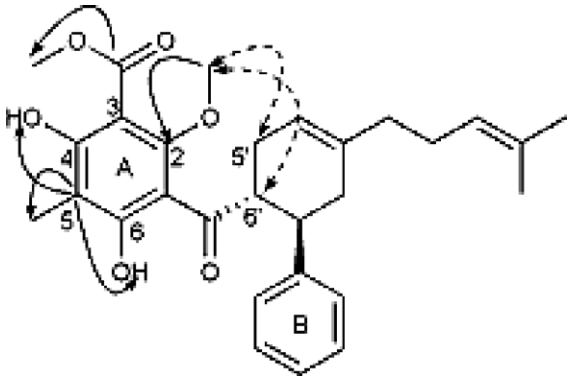

Fig. 4. Selected NOESY (dotted line) and HMBC (solid line) correlations for compound **3**.

differences in the resonances due to ring A. In the HMBC spectrum, a quaternary aromatic carbon at δ_C 108.6 was seen to correlate with the aromatic methyl at δ_H 1.90 and with the two hydrogen-bonded phenolic protons at δ_H 12.06 and δ_H 12.23 (Fig. 4). This consideration suggested that the aromatic methyl was located in C-5 position in the aromatic ring A and a hydroxyl group was borne by C-4 and C-6, respectively. Moreover, the presence of the second chelated hydroxyl group at δ_H 12.06 allowed us to assign the aromatic methyl ester in C-3 to a chemical shift at (δ_H 4.03) for the methyl group correlating with the carbonyl ester at (δ_C 170.9) in the HMBC spectrum. Long range correlations in the HMBC spectrum permitted to assign the last substituent in ring A, the methoxy group at δ_H 3.79 in C-2. The presence of two methine carbons C-6′ and C-1′ in α and β position, respectively of carbonyl function suggested that the di-prenylated moiety was substituted in these positions. This was supported by NOE interactions between the aromatic methoxy group (δ_H 3.79) with H-5′ (δ_H 2.49) and H-6′ (δ_H 4.21) (Fig. 4). The relative stereochemistry at (C-1′) and (C-6′) was deduced from the *J*-value between H-1′ and H-6′ ($J = 11$ Hz) indicating a trans diaxial coupling in a normal half-chair conformation (Tuntiwachwuttikul et al., 1984). In order to distinguish the two possibilities of the attachment of the prenyl side chain, we have observed long range HMBC correlations between H-4′ (δ_H 5.55) and C-(5′) (δ_C 30.2)/C-(6′) (δ_C 51.5) which confirmed the attachment of the prenyl unit to C-3′ like (±)-nicolaiodesin C (Gu et al., 2002). Compound **3** was determined as a racemate on the basis of the optical rotation profile, the structure shown in Fig. 4 is one of the two possible representations of the relative stereochemistry. Thus the structure of compound **3** was assigned as (1′*R**,6′*R**)-(4,6-dihydroxy-5-methyl-3-methylester-2-methoxyphenyl)-(3′-isohexenyl-1′-phenylcyclohex-3′-enyl) methanone and named hostmanin D. According to Shibata et al. (2000), these compounds could be conceivably derived from a non-enantioselective Diels–Alder reaction between the related chalcone and the acyclic monoterpene myrcene. Although numerous compounds containing cyclohexene moiety have been found in nature (Gu et al., 2002; Pancharoen et al., 1987; Shibata et al., 2000; Tuntiwachwuttikul et al., 1984), this constitutes the first report of this type of compound isolated in the genus *Piper*. The structures of the known compounds **4**–**8** were determined by comparison of their physical and spectroscopic features with those reported in the literature and identified as 2′,6′-dihydroxy-4′-methoxydihydrochalcone (Burke and Nair, 1986), linderatone (Ichino et al., 1990), strobopinin (Mayer, 1990), aduncin E (Orjala et al., 1993) and (−)-methyllinderatin (Ichino, 1989), respectively. When tested *in vitro* against *Plasmodium falciparum* (Table 3), at least two compounds (**5** and **8**) were considered to be potentially interesting. The dihydrochalcone **8** was as active as observed for similar molecules (Froelich et al., 2005). When tested on human

Table 3
Biological activity (μM) of the compounds **1**–**8**

	P. falciparum		Human cells	CAR FcB1[a]	CAR F32[b]
	F32 (2)[c]	FcB1 (2)	MCF7 (3)		
1	101.27 ± 4.56[d]	125.23 ± 28.05	242.64 ± 3.46	1.9	2.4
2	68.4 ± 3.33	79.01 ± 11.67	233.49 ± 3.33	2.9	3.4
3	55.44 ± 7.40	60.67 ± 8.89	207.12 ± 2.96	3.4	3.7
4	12.69 ± 0.26	16.91 ± 2.60	27.51 ± 5.23	1.63	2.2
5	10.33 ± 0.18	15.06 ± 9.38	52.29 ± 1.80	3.4	5.1
6	168.52 ± 23.57	366.48 ± 4.98	366.67 ± 5.23	1.0	2.2
7	126.75 ± 4.21	233.49 ± 3.33	233.49 ± 3.3	1.0	1.82
8	5.64 ± 1.04	5.27 ± 2.24	68.63 ± 10.4	13.0	12.2
CQ[e]	60×10^{-3}	145×10^{-3}	ND	ND	ND
Dox[f]	ND	ND	0.4	ND	ND

[a] CAR cytotoxic/antiplasmodial (FcB1) ratio.
[b] CAR cytotoxic/antiplasmodial (F32) ratio.
[c] Number of independent experiment.
[d] Means ± SD.
[e] CQ, chloroquine positive control for *P. falciparum* inhibition.
[f] Dox, doxorubicin; positive control for MCF7 inhibition.

Table 4
In vivo activity of (−)-methyllinderatin **8** against *P. vinckei petteri* in mice

Treatment	Dose ($mg^{-1}\ kg^{-1}\ day^{-1}$)	*n* [a]	Mean (±SD) parasitaemia[b]	Reduction of parasitaemia [b] (%)	Mean survival time (day)
Control		10	38.1 ± 3.7		7.7 ± 1.3
8	20	5	7.8 ± 6.7	80	11.1 ± 1.59
CQ[c]	10	5	0	100	>21
	1	5	33 ± 14.4	13	7.1 ± 1.1

[a] Number of mice by group.
[b] At day 4.
[c] Chloroquine.

cell lines, the most efficient compound (**8**) against *P. falciparum* had a CAR (cytotoxicity antiplasmodial ratio) higher than 10, a value that allowed to perform an *in vivo* test (Likhitwitayawuid et al., 1993). When tested *in vivo* against *P. vinckei petteri*, a clear decrease in parasitaemia (80% at day 5) was observed for the mice treated with **8** when compared to control mice (Table 4). The survival time was only of 3.5 additional days when compared to control thus a complete cure was not achieved at the dose tested.

3. Experimental

3.1. General experimental procedures

Melting points were determined on an Electrothermal 9100 apparatus. Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a sodium lamp ($\lambda = 589$ nm) in a 1 cm microcell. The UV spectra were recorded on an Anthelie 2 Advanced spectrometer. IR spectroscopy was performed on a Perkin–Elmer Paragon 1000 FT-IR spectrophotometer. NMR (500 and 400 MHz for ^{1}H NMR, 125 and 100 MHz for ^{13}C NMR) were obtained with $CDCl_3$ and DMSO-d_6 as solvent on a Bruker Avance 500 equipped with a TBI *z*-gradient 5 mm probe and a Bruker ARX 400 spectrometer. 1D and 2D (COSY, HSQC, HMBC, NOESY) NMR experiments were performed at 298 K using standard pulse sequences. Chemical shifts (δ) are given in ppm relative to TMS with coupling constant (*J*) reported in Hz. APCI-MS (positive and negative-ion mode) spectra were recorded on an LCQ DecaXPmax Thermo Electron mass spectrometer. HRESI-MS spectra (positive and negative-ion mode) were obtained on a Q-Tof Ultima (Waters) apparatus. Column chromatography and medium-pressure column chromatography were performed on silica gel 60 SDS 70–200 μm and 6–35 μm, respectively. Reversed-phase chromatographies were accomplished with RP-18 Mega Bond Elut® Varian cartridge and RP-18 (40–60 μm, AIT). Analytical and preparative HPLC were performed using μBondapack C_{18} column (10 μm, 3.9 × 300 mm, Waters) and Dynamax microsorb C_{18} column (5 μm, 10 × 250 mm, Varian) with a UV-DAD Hitachi L 7455 as detector. TLC was carried out on precoated silica gel 60 F_{254} aluminium plates (Merck). Spots were detected under UV (254 and 366 nm) before spraying with diphenylborate reagent or a vanillin sulfuric solution followed by heating the plate at 110 °C. All solvents were spectral grade or distilled from glass prior to use.

3.2. Plant material

Leaves of *Piper hostmannianum* var. *berbicense* (Miq.) C. DC. were collected in French Guiana in the Saint Elie tropical rain forest and identified by Dr Ricardo Callejas-Posada R (Univ Antioquia, Inst Biol, Fac Ciencias Exactas & Nat, Apartado Postal 1226, Medellin, Colombia, callejas@matematicas.udea.edu.co). This sampling spot is a permanent investigation area containing up to 800 identified trees. A herbarium voucher specimen (1016) was deposited at the IRD herbarium of Cayenne (CAY).

3.3. Extraction and isolation

Air-dried and powdered leaves of *P. hostmannianum* var. *berbicense* (500 g) were successively percolated with *n*-hexane (10 l), chloroform (20 l) and methanol (25 l). The dried *n*-hexane extract was found to exhibit the highest antiplasmodial activity with an $IC_{50} = 8$ μg/ml against the growth of chloroquine-resistant strain (FcB1). The isolation process was guided throughout by the results of the antiplasmodial activity and bioautographic TLC assays. After evaporation of the solvent under reduced pressure, the oily residue (15 g) was subjected to column chromatography (7.5 × 120 cm) on silica gel (400 g, 70–200 μm) and sequentially eluted with *n*-hexane, ethyl acetate and methanol to afford 14 fractions (500 ml each). Fractions F1 and F3 eluting with *n*-hexane–ethyl acetate 75:25 proved to be the most active in the antiplasmodial assay. Fraction F1 (4.9 g) was further purified by silica gel (150 g, 6–35 μm) medium-pressure column chromatography (3.5 × 17.5 cm) using a mixture of *n*-hexane–ethyl acetate of increasing polarity as eluent to give 13 fractions (150 ml each). The purification of the most potent fractions F1.2, F1.3, F1.4 was further investigated. From F1.2 (1.35 g), linderatone **5** (10 mg) and compound **1** (20 mg) were obtained after repeated purification on an RP-18 (30 g, 40–60 μm) medium-pressure column (2.5 ×

20 cm) chromatographic separation eluted with MeOH–H_2O 80:20. Fraction F1.3 (192 mg) was purified by chromatography on a reversed-phase silica gel cartridge (Varian®, Mega Bond Elut) (MeOH–H_2O 85:15) followed by a semi-preparative HPLC (RP-18 at a 3 ml/min flow rate; elution from 80% to 100% MeOH in H_2O over 10 min; 100% MeOH during 10 min; finally returning to the initial condition) to yielded compounds **2** (1.7 mg), **3** (1.5 mg), and adunctin E (**7**) (1.8 mg). From F1.4 (167 mg), (−)-methyllinderatin **8** (30 mg) was obtained after purification on a reversed-phase silica gel cartridge (Varian®, Mega Bond Elut) (MeOH–H_2O 85:15). 2′,6′-Dihydroxy-4′-methoxydihydrochalcone **4** (10 mg) and strobopinin **6** (4 mg) were isolated from fraction F3 (1.03 g) after a Sephadex® LH-20 column (2.5 × 20 cm) using $CHCl_3$ as eluting solvent followed by RP-18 column cartridge (Varian®, Mega Bond Elut) separation (MeOH–H_2O 60:40) and semi-preparative HPLC (RP-18 at a 3 ml/min flow rate of MeOH–H_2O 60:40).

3.4. Hostmanin A rel-(1″R,4″R,5″R)-2′-hydroxy-4′-methoxy-5′,6′-O-(4-isopropyl-1-methyl-cyclohexane-1-O,5-yl)dihydrochalcone (**1a**) and hostmanin B rel-(1″R,4″S,5″R)-2′-hydroxy-4′-methoxy-5′,6′-O-(4-isopropyl-1-methyl-cyclohexane-1-O,5-yl)dihydrochalcone (**1b**)

Yellow prisms: mp 58–60 °C (from MeOH); $[\alpha]_D^{25}$ − 31 (MeOH, *c* 0.15); UV λ_{max}^{MeOH} (log ε) 234 (3.00), 292 (3.15) nm; IR $\upsilon_{max}^{CHCl_3}$ 3425 (chelated OH), 1616 (C=O), 1585 (aromatic ring) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$), see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; APCI *m/z* 407 $[M-H]^+$; HRESIMS *m/z* 407.2242 (calcd. 407.2222) for $C_{26}H_{32}O_4$.

3.5. Hostmanin C rel-(1″R,4″R,5″S,6″S)-2′-hydroxy-4′-methoxy-5′,6′-O-(4-isopropyl-1-methyl-cyclohexan-1-ol-5,6-O-yl)dihydrochalcone (**2**)

White amorphous powder: $[\alpha]_D^{25}$ + 4 (MeOH, *c* 0.17); UV λ_{max}^{MeOH} (log ε) 232 (3.70), 285 (3.78) nm; IR $\upsilon_{max}^{CHCl_3}$ 3390 (*br* OH), 1633 (C=O), 1600, 1580 (aromatic ring), 1215 (C–OH) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; APCI *m/z* 423 $[M-H]^+$; HRESIMS *m/z* 425.2286 (calcd. 425.2328) for $C_{26}H_{32}O_5$.

3.6. Hostmanin D rel-(1′R*,6′R*)-(4,6-dihydroxy-5-methyl-3-methylester-2-methoxyphenyl)-(3′-isohexenyl-1′-phenylcyclohex-3′-enyl) methanone (**3**)

Yellow oil: $[\alpha]_D^{25}$ 0 (MeOH, *c* 0.1); UV λ_{max}^{MeOH} (log ε) 232 (3.23), 292 (3.52) nm; IR $\upsilon_{max}^{CHCl_3}$ 3500 (*br* OH), 1700 (O–C=0), 1616 (C=O), 1580 (aromatic ring) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; APCI *m/z* 477 $[M-H]^+$; HRESIMS *m/z* 479.2367 (calcd. 479.2434) for $C_{29}H_{34}O_6$.

3.7. 2′-6′-Dihydroxy-4′-methoxydihydrochalcone (**4**)

Colourless plates: mp 175 °C (from CH_2Cl_2) (Burke and Nair, 1986), 174–175 °C; UV λ_{max}^{MeOH} 232 (4.94) 287 (5.08) nm; IR $\upsilon_{max}^{CHCl_3}$ 3264 (*br*, OH), 1646 (C=O), 1600, 1526 (aromatic ring) cm^{-1}; APCI *m/z* 271 $[M-H]^+$; ^1H NMR and ^{13}C NMR (Orjala et al., 1994).

3.8. Linderatone (**5**)

Amorphous powder: $[\alpha]_D^{25}$ − 32.4 ($CHCl_3$, *c* 0.5); UV λ_{max}^{MeOH} (log ε) 295 (3.98), 335 (3.57); IR $\upsilon_{max}^{CHCl_3}$ 3435 (*br* OH), 1635 (C=O), 1620, 1580 (aromatic ring) cm^{-1}; APCI *m/z* 391 $[M+H]^+$; ^1H NMR and ^{13}C NMR (Ichino et al., 1990).

3.9. Strobopinin (**6**)

Solid: mp 228–230 °C (from Et_2O) (Mayer, 1990) 223–232 °C; $[\alpha]_D^{25}$ − 90.4($CHCl_3$, *c* 0.05); UV λ_{max}^{MeOH} (log ε) 295 (3.61), 335 (3.43) nm; IR $\upsilon_{max}^{CHCl_3}$ 3125 (chelated OH), 1635 (C=O), 1620, 1587 (aromatic ring) cm^{-1}; APCI *m/z* 269 $[M-H]^+$; ^1H NMR and ^{13}C NMR (Mayer, 1990).

3.10. Adunctin E (**7**)

White amorphous powder: $[\alpha]_D^{25}$ + 16.3 (MeOH, *c* 0.65); UV λ_{max}^{MeOH} (log ε) 232 (3.70), 285 (3.78) nm; IR $\upsilon_{max}^{CHCl_3}$ 3390 (*br* OH), 1633 (C=O), 1600, 1580 (aromatic ring), 1215 (C–OH) cm^{-1}; APCI *m/z* 423 $[M-H]^+$; ^1H NMR and ^{13}C NMR (Orjala et al., 1993).

3.11. (−)-Methyllinderatin (**8**)

Colourless oil: $[\alpha]_D^{25}$ − 40.1($CHCl_3$, *c* 0.1); UV λ_{max}^{MeOH} (log ε) 211 (4.72), 289 (4.54) nm; IR $\upsilon_{max}^{CHCl_3}$ 3359 (*br* OH), 1623 (C=O), 1580, 1450 (aromatic ring) cm^{-1}; APCI *m/z* 407 $[M-H]^+$; ^1H NMR and ^{13}C NMR (Ichino, 1989).

3.12. Plasmodium in vitro culture and antiplasmodial activity

Parasites were cultured according to the method described by Trager and Jensen (Trager and Jensen, 1976) with modifications described by Benoit et al. (1995). The cultures were synchronized every 48 h by 5% D-sorbitol lysis (Lambros and Vanderberg, 1979) (Merck, Darmstadt, Germany). The F32-Tanzania strain was considered as a chloroquino-sensitive strain (chloroquine IC_{50}: 60 nM), and FcB1-Columbia was considered as chloroquino-resistant strain (chloroquine IC_{50}: 145 nM). *In vitro* antimalarial activity testing was performed by [^{3}H]-hypoxanthine (Amersham-France) incorporation as described by Desjardins et al. (1979) with modifications (Valentin et al., 1997).

3.13. In vitro cytotoxicity evaluation

Human breast adenocarcinoma (MCF-7) cells were cultured in DMEM culture media containing 2 mM L-glutamine (Cambrex, Emerainville, France) supplemented with 1% fetal calf serum (FCS) (Cambrex) and incubated under standard conditions (37 °C, 5% CO_2). All experiments were carried out using cells in the exponential phase of growth. Cells were trypsinized, resuspended in DMEM containing 10% FCS and seeded (200,000 cells/ml) in 96-well plates. After 24 h the medium was replaced by fresh medium containing the compounds at concentrations ranging from 1 to 100 μg/ml. At the end of the treatment (48 h) cell viability was evaluated by measuring the activity of the mitochondrial enzyme succinate deshydrogenase. The medium was replaced by 50 μl of a sodium 2,3–bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt (XTT) (Sigma, Saint Quentin Fallavier, France) in water solution (0.5 mg/ml), and cells were incubated for 180 min. The reaction was stopped by addition of a SDS solution (10% in water). XTT was converted to a formazan product, detected by spectrophotometry at 450 nm. IC_{50} values were determined graphically from the dose–response curves (Tabbi et al., 2002).

3.14. In vivo antiplasmodial activity testing

In order to test the *in vivo* antimalarial activity, a 4-day-suppressive assay was performed on CD female mice, using *P. vinckei petteri* (Peters, 1965). Mice (mean body weight: 20 ± 2 g) were infected with 10^6 infected red blood cells in RPMI on day 0. Groups of five mice were treated intraperitonally from day 0–3 with one dose (20 mg/kg) of the most efficient drug **8**. On day 4, Giemsa stained smears were made for each mouse after tail blood sampling. Parasitaemia was estimated by visual counting of at least 5000 erythrocytes. Controls were mice treated with RPMI alone or chloroquine (1 and 10 mg/kg). The inhibition percentage was calculated with the following formula: (control parasitaemia – parasitaemia with drugs)/ (control parasitaemia) X 100. Mice were maintained under institutional animal guidelines.

3.15. X-ray crystallographic analysis of *1*

$C_{26}H_{32}O_4$, $M = 408.52$, monoclinic, $P2_1$, $a = 11.739(1)$ Å, $b = 16.054(1)$ Å, $c = 12.667(1)$ Å, $\beta = 111.718(2)°$, $V = 2217.9(3)$ Å^3, $Z = 4$, $T = 173(2)$ K. Nine thousand nine hundred and sixteen reflections (6272 independent, $R_{int} = 0.0438$) were collected. Largest electron density residue: 0.138 e Å^{-3}, R_1 (for $I > 2\sigma(I)$) = 0.0494 and $wR_2 = 0.0976$ (all data) with $R_1 = \Sigma||F_o| - |F_c||/\Sigma\ |F_o|$ and $wR_2 = (\Sigma w(F_o^2 - F_c^2)^2 / \Sigma w(F_o^2)^2)^{0.5}$. All the data for this structure was collected at low temperatures using an oil-coated shock-cooled crystal on a Bruker-AXS CCD 1000 diffractometer with Mo Kα radiation ($\lambda = 0.71073$ Å). The structure was solved by direct methods (SHELXS-97, (Sheldrick, 1990)) and all non-hydrogen atoms were refined anisotropically using the least-squares method on F^2 (SHELXL-97, Program for Crystal Structure Refinement, G.M. Sheldrick, University of Göttingen 1997).

4. Supplementary material

The crystallographic data for the structures reported in this paper has been deposited with the Cambridge Crystallographic Data centre as supplementary publication (Deposition No. CCDC 624770). This data can be obtained free of charge, by request to the Director, via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

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