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New antimalarials with a triterpenic scaffold from *Momordica balsamina*

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ABSTRACT

Bioassay-guided fractionation of the methanol extract of *Momordica balsamina* led to the isolation of three new cucurbitane-type triterpenoids, balsaminols C–E (**1**–**3**). Their structures were elucidated on the basis of spectroscopic methods including 2D NMR experiments (COSY, HMQC, HMBC and NOESY). Balsaminols C–E, together with ten cucurbitacins isolated from the same plant (**4**–**13**), were evaluated for their antimalarial activity against the *Plasmodium falciparum* chloroquine-sensitive strain 3D7 and the chloroquine-resistant clone Dd2. Most of the compounds displayed antimalarial activity. Compounds **9** and **12** revealed the highest antiplasmodial effects against both strains (IC_{50} values: 4.6, and 7.4 μM, 3D7, respectively; 4.0, and 8.2 μM, Dd2, respectively). Structure–activity relationships are discussed. Furthermore, the preliminary toxicity toward human cells of compounds **1**–**5** and **9** was investigated in breast cancer cell line (MCF-7). Compounds were inactive or showed weak toxicity (IC_{50} values >19.0).

1. Introduction

Malaria is one of the most common infectious diseases in over 100 countries in Africa, Southeast Asia, and South America, causing more than one million deaths every year.[1] The emergence of multidrug-resistant *Plasmodium* strains, particularly *Plasmodium falciparum*, represents a major problem for both prophylaxis and treatment of malaria. In order to overcome this disease, new therapeutic agents based on new mechanisms of action or with new structures became necessary.[2] The antimalarial potential of drugs derived from plants has been widely illustrated by quinine, artemisinin and their derivatives, the most important antimalarials currently available to treat malaria. Therefore, taking into account the crucial role that plant-derived compounds have played in drug discovery and development, the isolation of new bioactive compounds or leads from medicinal plants seems to be a very promising approach.[3–5]

Momordica balsamina L. (Cucurbitaceae), a vegetable used as food, has also been widely used in traditional medicine, particularly for the treatment of fever and malaria in Mozambique and South Africa.[6,7] Previously, as a part of our search for bioactive compounds from medicinal African plants,[8–10] we have carried out a preliminary screening of different extracts from fifteen plant species for their antimalarial activity against the 3D7 chloroquine-sensitive *P. falciparum* strain. The EtOAc extract of *M. balsamina* was found to be the most active one,[11] corroborating previous in vitro and in vivo antimalarial studies on this species.[7,12] Based on those results, a bioassay-guided fractionation of the aerial parts of *M. balsamina* has carried out, providing the cucurbitane-type triterpenoids **4**–**13** (Fig. 1).[13,14] Some of the isolated compounds were found to be strong multidrug resistance reversers in cancer cells.[13] In this paper, we are reporting the isolation and structure elucidation of three new cucurbitacins (**1**–**3**) from the same plant. Moreover, compounds **1**–**13** were evaluated for their antimalarial activity against two different *P. falciparum* strains (3D7 and Dd2). The cytotoxicity of compounds **1**–**5** and **9** against human breast cancer cells (MCF-7) is also reported.

2. Results and discussion

2.1. Structure elucidation of compounds

The air-dried powdered aerial parts of *M. balsamina* were exhaustively extracted with methanol. A bioassay-guided fractionation of the EtOAc soluble part of the methanol extract yielded the new compounds **1**–**3**.

Compound **1**, named balsaminol C, was obtained as an amorphous white powder. Its molecular formula was assigned as $C_{30}H_{46}O_4$ by HR-CIMS spectrum, which showed a pseudomolecular

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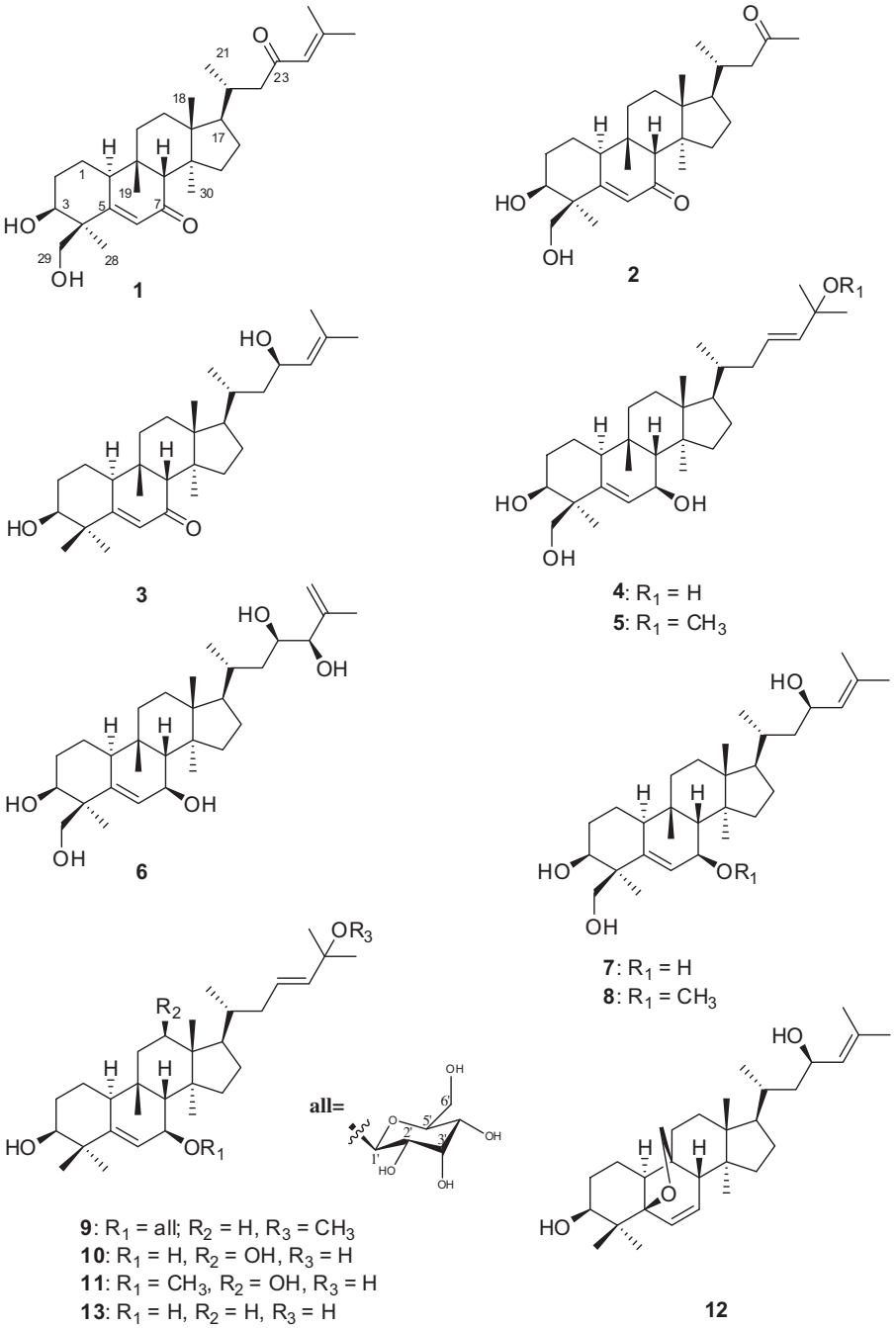

Figure 1. Chemical structures of compounds **1**–**13**.

ion at *m/z* 471.3466 $[M+1]^+$ (calcd for $C_{30}H_{47}O_4$, 471.3474). The IR spectrum of **1** showed absorption bands at 3384 and 1641 cm^{-1}, characteristics of the hydroxyl function and conjugated carbonyl groups, respectively. The UV spectrum exhibited an absorption maximum at 255 nm, corroborating the presence of α,β-unsaturated ketone groups. In the ^{13}C NMR spectrum, due to mesomeric effects, the existence of two relatively high-field carbonyl signals (δ_C 205.5 and 203.9), together with two remarkable downfield sp^2 carbons (δ_C 170.6 and 157.1), was consistent with the mentioned unsaturated systems. The ^1H NMR spectrum of **1** (Table 1) displayed signals for four tertiary methyl groups (δ_H 0.91, 0.96, 0.98, and 1.11), two vinylic methyls (δ_H 1.91, and 2.12), one secondary methyl group at δ_H 0.92 (d, J = 7.0 Hz), and one diastereotopic methylene group bounded to oxygen at δ_H 3.72 and 3.90 (d, J = 10.9 Hz). In addition, a broad singlet of an oxygenated methine proton at δ_H 3.92, assignable to H-3 with an equatorial configuration ($J_{3eq,2ax} \cong J_{3eq,2eq}$), was also found. The ^1H NMR spectrum of **1** also provided evidence for two trisubstituted double bonds at δ_H 6.10 and 6.18. The ^{13}C NMR spectrum displayed 30 carbon resonances discriminated by a DEPT experiment as seven methyl groups, eight methylenes (one carbon bearing oxygen at δ_C 68.7), seven methines (including an oxygenated sp^3 carbon at δ_C 75.5 and two sp^2 carbons at δ_C 126.9, and 125.3) and eight quaternary carbons (the two mentioned sp^2 at δ_C 170.6, 157.1, and the carbonyl signals at δ_C 205.5 and 203.9). According to the molecular formula $C_{30}H_{46}O_4$, **1** contains eight degrees of unsaturation and therefore a tetracyclic triterpenoid scaffold, having two hydroxyl groups and two enone systems is proposed. Besides the characteristic hydroxyl at C-3, the location

Table 1
NMR data of compounds **1**–**3**, (MeOD, *J* in Hz)

Position	**1**		**2**		**3**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	1.77 m; 1.88 m	22.0	1.77 m; 1.88 m	22.0	1.79 m; 1.87 m	22.3
2	1.75 m; 1.99 m	29.8	1.75 m; 2.00 m	29.8	1.76 m; 2.02 m	29.8
3	3.92 br s	75.5	3.91 br s	75.5	3.62 br s	77.5
4	–	47.2	–	47.2	–	44.1
5	–	170.6	–	170.6	–	174.1
6	6.10 s	126.9	6.09 s	126.9	6.06 s	126.1
7	–	205.5	–	205.5	–	205.8
8	2.39 s	61.1	2.38 s	61.1	2.39 s	61.4
9	–	37.0	–	36.9	–	36.9
10	2.84 br d (11.3)	41.7	2.84 dd (2.7, 11.3)	41.7	2.84 m	41.9
11	1.47 m; 1.89 m	32.2	1.52 m; 1.89 m	32.2	1.51 m; 1.89 m	32.3
12	1.28 m; 1.63 m	30.9	1.30 m; 1.63 m	30.9	1.65 m; 1.86 m	31.1
13	–	47.0	–	47.0	–	47.0
14	–	49.7	–	49.7	–	49.8
15	1.03 m; 1.56 m	35.8	1.03 m; 1.37 m	35.8	1.03 m; 1.56 m	35.8
16	1.36 m; 1.99 m	29.0	1.33 m; 1.97 m	28.9	1.29 m; 1.90 m	29.0
17	1.57 m	51.3	1.55 m	51.1	1.52 m	51.7
18	0.96 s	15.9	0.95 s	15.8	0.95 s	15.9
19	0.98 s	28.2	0.98 s	28.2	0.98 s	28.3
20	2.04 m	34.9	2.04 m	34.1	1.56 m	33.9
21	0.92 d (7.0)	20.2	0.91 d (7.0)	20.2	1.01 d (7.0)	19.4
22	2.10 d (13); 2.54 m	52.7	2.19 m; 2.55 m	51.8	0.98 m; 1.66 m	45.6
23	–	203.9	–	212.2	4.42 td (3.2, 9.6)	66.6
24	6.18 s	125.3	2.11 s	30.6	5.17 d (8.2)	130.5
25	–	157.1	–	–	–	133.5
26	1.91 s	20.9	–	–	1.68 s	18.2
27	2.12 s	27.7	–	–	1.71 s	25.6
28	1.11 s	23.3	1.11 s	23.3	1.24 s	28.6
29a	3.72 d (10.9)	68.7	3.72 d (10.9)	68.7	1.17 s	26.0
29b	3.90 d (10.9)	–	3.90 d (10.9)	–	–	–
30	0.91 s	18.8	0.90 s	18.8	0.91 s	18.8

of an unusual hydroxyl group at C-29 was also indicated by the changes observed in NMR data for carbons and protons of ring A, when comparing with those found for cucurbalsaminol A.[14] In this way, the paramagnetic effects at C-29 ($\Delta\delta$ = +42.6 ppm; α-carbon), and C-4 ($\Delta\delta$ = +4.9 ppm; β-carbon), and the diamagnetic effects at C-3 and C-28 ($\Delta\delta$ = −1.9 and −5.4 ppm, respectively; γ-carbons) were in good agreement with the changes expected for the location of a hydroxyl group at C-29.[15] The clear deshielding of H-3 (δ_H 3.92), maybe due to intramolecular hydrogen bonding between the hydroxyl groups at C-29 and C-3, also corroborates the existence of free hydroxyl groups at C-29. This uncommon structural feature in cucurbitane-type triterpenoids was only found in compounds **4**–**8**.[13,14]

Analysis of the two-dimensional data, together with ^{1}H and ^{13}C NMR spectra, allowed the clear assignment of all carbon signals (Table 1). The ^{1}H–^{1}H COSY and HMQC experiments revealed the structure of the following spin-systems: –CH_2–CH(OH)– (A); –CH_2–CH–C=CH– (B); –CH(CH_3)–CH_2– (C) and –CH=C–(CH_3)$_2$– (D). The connection of these structural fragments and the location of the functional groups were determined on the basis of key long-range correlations, displayed in the HMBC spectrum (Fig. 2). In this way, the α,β-unsaturated carbonyl carbon at C-7 was supported by the $^2J_{C-H}$ correlation between the carbonyl signal (δ_C 205.5) and H-8 (δ_H 2.39). On the other hand, the presence of an enone system at the side chain was supported by the HMBC long-range hetero-correlations of both the carbons of the vinylic methyls (δ_C 20.9, 27.7), and the carbonylic group (δ_C 203.9) with the vinylic proton H-24 (δ_H 6.18). The ion peak at *m/z* 345 [M−side chain]$^+$, displayed by the EIMS, together with the ion at *m/z* 373 [M−$CH_2COCHC(CH_3)_2$]$^+$, arising from cleavage of the C-20–C-22 bond, confirmed the proposed structural feature for the side chain. The presence of a hydroxyl group at both C-3 and C-29 was supported by the key HMBC correlations of C-3 (δ_C 75.5) with H-29 (δ_H 3.72, 3.90), and Me-28 (δ_H 1.11), and between C-29 (δ_C 68.7) and Me-28. Moreover, the long-range HMBC correlations of C-5 (δ_C 170.6) with Me-28/H-29 corroborated the oxidation of Me-29. The position of the $\Delta^{5,6}$ double bond was unambiguously defined by the HMBC cross-peaks between C-10 (δ_C 41.7)/H-6, C-4/H-6, and C-8/H-6, together with the allylic correlation between H-6 and H-10, observed in the ^{1}H–^{1}H COSY spectrum.

The relative configuration of compound **1** was characterized by a NOESY experiment (Fig. 3), taking into account the coupling constants pattern and assuming an α orientation for H-10,[16] characteristic of cucurbitacins. In this way, the β-orientation of the hydroxymethyl at C-4 and the hydroxyl group at C-3 was supported by the NOE correlations between H-10/Me-28, and Me-28/H-3. The interactions between H-8/Me-19 and H-8/Me-18 supported the β-orientation of these protons. On the other hand, the cross peaks between H-10/Me-30 and Me-30/H-17 supported the usual α-orientation of Me-30 and H-17. These findings and comparison of ^{1}H NMR and ^{13}C NMR spectra of **1** with those of known compounds,[17,18] led us to formulate the structure of balsaminol C as cucurbita-5,24-diene-7,23-dione-3β,29-diol.

Compound **2**, named balsaminol D, was obtained as an amorphous white powder. In the low-resolution ESIMS data, a pseudomolecular ion [M+Na]$^+$ at *m/z* 453 was observed. Its molecular formula was determined as $C_{27}H_{42}O_4$, on the basis of the HR-ESI-TOFMS spectrum, which exhibited a pseudomolecular [M+H]$^+$ ion peak at *m/z* 431.3153 (calcd for $C_{27}H_{43}O_4$, 431.3156), indicating seven double bond equivalents. The IR spectrum showed absorption bands for hydroxyl groups (3375 cm^{-1}), an isolated ketone (1707 cm^{-1}), and a conjugated carbonyl group (1645 cm^{-1}), being the latter corroborated by the UV spectrum (λ_{max} = 252 nm). Comparison of NMR data of **2** (Table 1) with those of **1** (Table 1), revealed that the two compounds shared the same triterpenic nucleus, differing only in the side chains. Further, analysis of NMR data revealed that compound **2** has a *trinor*-cucurbit-5-ene-7,23-dione skeleton, without signals for C-25, C-26, and C-27. In

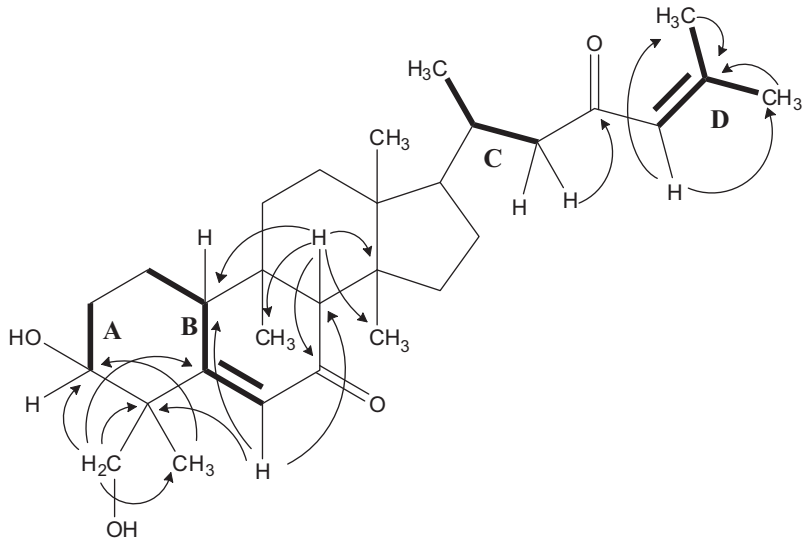

Figure 2. ^{1}H-spin systems (A–D) of compound **1** assigned by the HMQC and COSY experiments (—) and their connection by heteronuclear $^{2}J_{H-C}$ and $^{3}J_{H-C}$ correlations displayed in the HMBC spectrum (→).

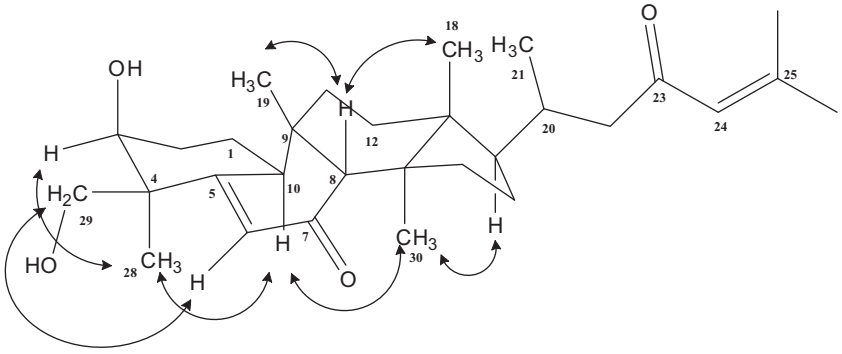

Figure 3. Key NOESY correlations of compound **1**.

this way, the ^{1}H and ^{13}C NMR data of the side chain of **2** showed resonances for one tertiary methyl group particularly deshielded (δ_H 2.11; δ_C 30.6), one secondary methyl group (δ_H 0.91, d, J = 7.0 Hz; δ_C 20.2), and a carbonyl group at δ_C 212.2. The placement of a carbonyl group at C-23 was supported by the $^{2}J_{C-H}$ correlation, observed in the HMBC spectrum, of C-23 (δ_C 212.2) with Me-24 (δ_H 2.11), and the $^{3}J_{C-H}$ correlation between C-22 (δ_C 51.8) and Me-24. The relative configuration of **2**, determined by a NOESY experiment, was found to be identical to that of compound **1**. Similarly, the cross-peaks of H-10 with Me-28 and Me-30, Me-28 with H-3, and Me-30/H-17 indicated the same α-orientation for these protons. Further NOE correlations between Me-19/H-8 and H-8/Me-18 corroborated the β-orientation of these protons. Therefore, compound **2** was elucidated as 25,26,27-*trinor*-cucurbit-5-ene-7,23-dione-3β,29-diol.

Compound **3**, named balsaminol E, was obtained as an amorphous white powder. Its molecular formula was assigned as $C_{30}H_{48}O_3$ based on the molecular ion at *m/z* 456.3606, exhibited by the HR-EIMS spectrum (calcd for $C_{30}H_{48}O_3$, 456.3603). The IR and the UV data were similar to those of compounds **1** and **2**. Therefore, these data, together with the NMR profile (Table 1), were indicative of a tetracyclic triterpenoid bearing a hydroxyl group at C-3 and an α,β-unsaturated carbonyl group at ring B, and differing from **1** and **2** in signals of ring A and side chain. When comparing the NMR data of compounds **3** and **1**, the presence of an extra singlet (δ_H 1.17) in the aliphatic region of the ^{1}H NMR spectrum of **3**, together with remarkable upfield shifts at C-29 and C-4 (α and β-carbons, respectively), and downfield shifts at C-3, C-5, and C-28, (γ-carbons), indicated that C-29 was not oxidized. Regarding the side chain, the spectroscopic data indicated the presence of a hydroxyl at C-23, previously also found in compounds **7**, and **8**.[14] The diamagnetic effect at C-23 ($\Delta\delta_C$ = −137.7 ppm, α-carbon), and the paramagnetic at C-24 ($\Delta\delta_C$ = +5.2 ppm, β-carbon), observed for compound **3** relatively to **1**, are in good agreement with the effects expected for the substitution of a carbonyl by a hydroxyl group. This feature was also indicated by the disappearance of one carbonyl signal in the ^{13}C NMR spectrum, along with the presence of one additional hydroxymethine carbon signal at δ_C 66.6, which exhibited a HMQC correlation with the oxymethine proton at δ_H 4.42 (td, J = 3.2, 9.6 Hz). The location of a hydroxyl group at C-23 was supported by the observation of HMBC correlations between C-24 (δ_C 130.5)/C-25 (δ_C 133.5) with H-23 (δ_H 4.42) and the vinylic methyls Me-26 and Me-27 (δ_H 1.68 and 1.71). The base peak in the low resolution EIMS spectrum at *m/z* 357 [M−CH_2CHO-HCHC$(CH_3)_2$]$^{+}$, together with the fragment at *m/z* 329 [M−side chain]$^{+}$ corroborated the structure of the side chain. The relative stereochemistry at C-23 was assigned as *R*, by comparison of its ^{13}C NMR data with those reported for some lanostane derivatives.[19,20] A strong NOE interaction between Me-21 and H-23, found in the NOESY spectrum, corroborated this assignment. The same *R* configuration was also found for compounds **7**, and **8**, which was supported in the former by a energy minimization

calculation of its 3D structure, that provided evidence for a preferred conformation of the side chain.[14] Hence, the structure of **3** was formulated as cucurbita-5,24-dien-7-one-3β,23(*R*),29-triol.

2.2. Biological activity

Balsaminols C–E (**1**–**3**), characterized above, and compounds **4**–**13**, previously isolated from the same plant,[13,14] were evaluated for their antimalarial activity against chloroquine-sensitive (3D7) and resistant (Dd2) *P. falciparum* strains. A standardized SYBR Green I-based fluorescence assay was used in the experiment.[21,22] SYBR Green I is one of the most sensitive stains available for detection of double-stranded DNA, due to its remarkable affinity and fluorescence enhancement caused by its interaction with the nucleic acid. The effects of compounds (**1**–**13**) against both strains of *P. falciparum*, expressed as IC_{50} values in μM and μg/mL, are summarized in Table 2, as well as their cytotoxic activity against breast cancer cells (MCF-7). Cytotoxic activity results of compounds **6**–**8** and **10**–**13** were taken from our previous work,[14] and included for calculation of selectivity index (SI). As can be observed in Table 2, most of the compounds displayed antimalarial activity. Balsaminoside A (**9**) and karavilagenin E (**12**) revealed the highest antimalarial activities against both strains (**9**, IC_{50} values: 4.6 and 4.0 μM, 3D7 and Dd2, respectively; **12**, IC_{50} values: 7.4 and 8.2 μM, 3D7 and Dd2, respectively). Compounds **1**, **3**–**8**, **10**, and **11** displayed a moderated antimalarial activity with IC_{50} values ranging from 13.2 to 20.4 μM against 3D7, and between 17.6 and 33.0 μM against Dd2 *P. falciparum* strain. Compounds **2** and **13** have shown no significant activity, mainly against the resistant strain. Concerning the toxicity toward human cells, the compounds were inactive or showed a weak toxicity (IC_{50} values >19.0 μM) against the human breast cancer cell line studied (Table 2). As shown in Table 2, a low selectivity index (SI) was found for all compounds (SI = cytotoxic IC_{50}/antiplasmodial IC_{50}). Nevertheless, it should be noted that in previous in vivo studies, the extracts of *M. balsamina* have shown very weak toxicity.[12]

When comparing the results obtained for compounds **1**–**13**, rings A and B seem to play an important role in the antiplasmodial activity. This feature can be illustrated by the significant difference of the IC_{50} values found for compounds **9** (4.6; 4.0 μM, 3D7 and Dd2, respectively) and **5** (18.7, and 19.2 μM, 3D7 and Dd2, respectively). In fact, compound **9**, which displayed the highest activity, only differs from **5**, in the substitution pattern of those rings, having compound **9** a sugar unit at C-7, instead of the free hydroxyl present at this position in compound **5**. The latter, also bears a hydroxyl at C-29, absent in compound **9**. These results are corroborated with those obtained for the set of compounds **3**, **7**, **8**, and **12**, which only differ in the functional groups at rings A and B as well. In fact, compounds **3**, **7** and **8** exhibited similar IC_{50} values, which were significantly different from those found for compound **12**, characterized by an ether linkage between C-19 and C-5 and a different position for the double bond.

Based on these results, and considering in vivo previous data,[12] we can conclude that the isolated compounds were responsible for the activity found in the extracts in the preliminary screening and may be interesting as leads for the development of new antimalarials. Our study also supports the use of *M. balsamina* against malaria in traditional medicine.

3. Experimental section

3.1. General experimental procedures

Optical rotations were obtained using a Perkin Elmer 241 polarimeter. UV spectra were taken on a Shimadzu UV 1240 spectrometer. IR spectra were determined on a FT-IR Nicolet Impact 400, and NMR spectra recorded on a Bruker ARX-400 NMR spectrometer (^1H 400 MHz; ^{13}C 100.61 MHz), using CD_3OD as solvent. ESIMS were taken on a Micromass Quattro micro API and HR-ESITOFMS on a Bruker-Microtof ESITOF (Biotof II Model, Brucker). EIMS, HR-EIMS, HR-CIMS were recorded on a Micromass Autospec spectrometer. Column chromatography was carried out on SiO_2 (Merck 9385). TLC were performed on precoated SiO_2 F254 plates (Merck 5554 and 5744) and visualized under UV light and by spraying with sulfuric acid–methanol (1:1) followed by heating. HPLC was carried out on a Merck-Hitachi instrument, with UV detection (210 and 220 nm), using a Merck LiChrospher 100 RP-18 (10 μm, 250 × 10 mm) column.

3.2. Plant material

The aerial parts of *M. balsamina* L. were collected in Gaza, Mozambique, in August 2006. The plant material was identified by the botanist Dr. Silva Mulhovo, and a voucher specimen (30 SM) has been deposited at the herbarium (LMA) of the Instituto de Investigação Agronómica, Maputo, Mozambique.

3.3. Extraction and isolation

Dried aerial parts of *M. balsamina* (1.2 kg) were powdered and exhaustively extracted with methanol (11 × 8 L) at room temperature. The MeOH extract was evaporated to afford a residue (280 g),

Table 2
Antimalarial activity, cytotoxicity, and selectivity index of compounds **1**–**13**

Compound No.	IC_{50} ± SD					Selectivity index[a]	
	P. falciparum 3D7		*P. falciparum* Dd2		MCF7 cells	MCF7/3D7	MCF7/Dd2
	μM	μg/mL	μM	μg/mL	μM		
1	19.6 ± 1.5	9.2 ± 0.7	22.4 ± 4.0	10.5 ± 1.9	70.5 ± 2.9	3.6	3.2
2	25.9 ± 5.6	11.1 ± 2.4	45.6 ± 1.9	19.6 ± 0.8	>133.3	>5.1	>2.9
3	20.4 ± 2.1	9.3 ± 1.0	19.6 ± 4.6	9.0 ± 2.1	44.2 ± 3.9	2.2	2.3
4	19.1 ± 3.4	9.0 ± 1.6	27.3 ± 6.1	12.9 ± 2.9	65.1 ± 1.6	3.4	2.4
5	18.7 ± 1.6	9.1 ± 0.8	19.2 ± 2.7	9.4 ± 1.3	34.0 ± 0.1	1.8	1.8
6	14.6 ± 1.3	7.2 ± 0.6	33.0 ± 6.3	16.2 ± 3.1	47.0 ± 0.4	3.2	1.4
7	17.1 ± 0.4	8.1 ± 0.2	23.5 ± 4.9	11.4 ± 2.4	40.0 ± 1.3	2.3	1.7
8	15.2 ± 5.4	7.4 ± 2.6	17.9 ± 3.7	8.7 ± 1.8	31.0 ± 3.7	2.0	1.7
9	4.6 ± 0.5	2.9 ± 0.3	4.0 ± 0.5	2.5 ± 0.3	19.0 ± 0.8	4.2	4.7
10	13.2 ± 0.8	6.3 ± 0.4	17.6 ± 0.7	8.3 ± 0.3	>133.3	>10.1	>7.6
11	17.7 ± 1.3	8.6 ± 0.6	28.3 ± 4.6	13.8 ± 2.2	55.4 ± 2.9	3.1	2.0
12	7.4 ± 0.8	3.3 ± 0.4	8.2 ± 0.7	3.8 ± 0.3	30.7 ± 2.3	4.2	3.7
13	30.6 ± 6.4	14.0 ± 2.4	50.1 ± 0.1	23.0 ± 0.04	45.3 ± 0.7	1.5	0.9
CQ	16 nM	–	200 nM	–	–	–	–

[a] Selectivity index (SI) = cytotoxic IC_{50}/antiplasmodial IC_{50}.

which was suspended in H_2O (1 L) and extracted with EtOAc (9 × 0.5 L). The EtOAc residue (85 g) was suspended in MeOH/H_2O (9:1; 1 L), and extracted with *n*-hexane (5 × 0.5 L) for removal of waxy material that was not further studied. The remaining extract was evaporated under vacuum (40 °C), yielding a residue (45 g) that was chromatographed over silica gel (1 kg), using mixtures of *n*-hexane–EtOAc (1:0–0:1) and EtOAc–MeOH (19:1–0:1) as eluents to obtain six fractions (Fr 1–6), which were combined according to TLC analysis. Fr 3 (5.3 g) (*n*-hexane–EtOAc, 1:1–0:1) was subjected to a silica gel column chromatography with mixtures of *n*-hexane–EtOAc, to yield several fractions. The residue (1.1 g) eluted with *n*-hexane–EtOAc (2:3–1:1) was chromatographed twice, using gradients of CH_2Cl_2–acetone, and further purified by preparative TLC (*n*-hexane–EtOAc, 2:3) to yield 10 mg of compound **3**. The residue (843 mg) eluted with *n*-hexane–EtOAc (3:17–0:1) was repeatedly chromatographed on silica gel, using, as eluents, mixtures of CH_2Cl_2–acetone of increasing polarity. Further purification by HPLC afforded 10 mg of compound **1** (220 nm, MeOH/H_2O 4:1, 5 mL/min, t_R = 10 min). Fr 4 (3.25 mg), eluted with mixtures of *n*-hexane–EtOAc (1:19–0:1), was chromatographed over SiO_2 with mixtures of *n*-hexane–EtOAc, and CH_2Cl_2–MeOH and purified by HPLC (210 nm, MeOH/H_2O, 73:27; 5 mL/min, R_t 9 min) to give 17 mg of compound **2**.

3.3.1. Balsaminol C, cucurbita-5,24-diene-7,23-dione-3β,29-diol (1)

Amorphous, white powder; $[\alpha]_D^{26}$ +84 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.9), 219 (3.8), 239 (3.8), 255 (3.8); IR (KBR) ν_{max} 3384, 1679, 1641, 1449, 1379, 1300, 1020 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z*: 470 $[M]^+$ (4), 373 $[M-CH_2COCHC(CH_3)_2]^+$ (63), 345 [M−side chain]$^+$ (9), 325 (100), 298 (18), 175 (41), 125 (18), 83 (57); HR-CIMS *m/z*: 471.3466 $[M+1]^+$ (calcd for $C_{30}H_{47}O_4$, 471.3474).

3.3.2. Balsaminol D, 25,26,27-*trinor*-cucurbit-5-ene-7,23-dione-3β,29-diol (2)

Amorphous, white powder; $[\alpha]_D^{26}$ +103 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.9), 226 (3.8), 238 (3.8), 252 (3.8); IR (KBR) ν_{max} 3375, 1707, 1645, 1459, 1382, 1182, 1030 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z*: 453 $[M+Na]^+$; HR-ESI-TOFMS *m/z*: 431.3153 $[M+H]^+$ (calcd for $C_{27}H_{43}O_4$, 431.3156).

3.3.3. Balsaminol E, cucurbita-5,24-dien-7-one-3β,23(*R*)-diol (3)

Amorphous, white powder; $[\alpha]_D^{26}$ +98 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.9), 241 (3.9); IR (KBR) ν_{max} 3404, 2952, 1717, 1642, 1459, 1381, 1298 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* (rel int.): 456 $[M]^+$ (12), 438 $[M-H_2O]^+$ (22), 423 (7), 357 $[M-CH_2CHOHC(CH_3)_2]^+$ (100), 329 [M−side chain]$^+$ (40), 287 (6), 252 (6), 219 (12), 207 (26), 189 (40), 166 (22), 149 (16), 133 (26), 109 (31), 81 (23); HREIMS *m/z*: 456.3606 $[M]^+$ (calcd for $C_{30}H_{48}O_3$, 456.3603).

3.4. Biological assays

3.4.1. Antimalarial assay

Human malaria parasites were cultured as previously described by Trager and Jensen (1976), with minor modifications.[23] Briefly, 3D7 and Dd2 *P. falciparum* strains were cultivated in recently collected erythrocytes as host cells in RPMI 1640 medium (Gibco) containing 25 mM HEPES (Sigma) and 6.8 mM hypoxanthine (Sigma) supplemented with 10% AlbuMAX II (Invitrogen). Cultures were maintained at 37 °C under an atmosphere of 5% O_2, 3–5% CO_2, and N_2. The antimalarial activity of the compounds was determined by a fluorometric method using SYBR Green I.[21,22] In brief, stock solutions of the samples were prepared in DMSO (10 mg/mL), and were diluted to give a series of concentrations ranging from 0.156 to 100 μg/mL. Fifty microliters of each testing concentration, together with 50 μL of a 1% red blood parasitized cell suspension with ring stages and 2% hematocrit were distributed in duplicate, into each of the 96-well plates. Plates were incubated for 48 h at 37 °C. After, 100 μL of SYBR Green I in lysis buffer (Tris 20 mM; pH 7.5, EDTA—5 mM, saponin—0.008%; wt/vol, Triton X-100—0.08%; vol/vol, and 0.2 μL of SYBR Green I/mL of lysis buffer) was added to each well. Plates were covered, mixed and incubated in the dark at room temperature for 1 h. Fluorescence intensity was measured on a fluorescence multiwell plate reader, Anthos venyth 3100 (Alfagene) excitation and emission wavelengths of 485 and 535 nm, respectively. Values were expressed in relative fluorescence units. Analysis of the results obtained and IC_{50} determination were performed with HN-NonLineV1.1 (H. Noedl, 2001) software.

3.4.2. In vitro cytotoxicity assay

Human breast cancer MCF-7 cell line was cultured in RPMI 1640 medium supplemented with 10% heat inactivated horse serum, L-glutamine (2 mM), and antibiotics, in a humidified atmosphere of 5% CO_2 at 37 °C. The effects of increasing concentrations of the compounds on cell growth were tested in 96-well flat-bottomed microtiter plates. The compounds were diluted in a volume of 50 μL medium. Then, 2×10^4 cells in 0.1 mL of medium were added to each well, with the exception of the medium control wells. The culture plates were further incubated at 37 °C for 24 h. At the end of the incubation period, 15 μL of MTT (thiazolyl blue, Sigma, St. Louis, MO, USA) solution (from a 5 mg/mL stock) was added to each well. After incubation at 37 °C for 4 h, 100 μL of sodium dodecyl sulfate (SDS) (Sigma, St. Louis, MO, USA) solution (10%) was measured into each well and the plates were further incubated at 37 °C overnight. The cell growth was determined by measuring the optical density (OD) at 550 nm (ref. 630 nm) with a Dynatech MRX vertical beam ELISA reader. Inhibition of cell growth (as a percentage) was determined according to the formula:

$$100 - \left[\frac{\text{OD sample} - \text{OD medium control}}{\text{OD cell control} - \text{OD medium control}}\right] \times 100$$

where IC_{50} is defined as the inhibitory dose that reduces the growth of the compound-exposed cells by 50%. The IC_{50} values are expressed as means ± SD from three experiments.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.05.054.

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