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New pentacyclic triterpene saponins with strong *anti*-leishmanial activity from the leaves of *Maesa balansae*

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Abstract—Six new triterpene saponins bearing an oxygen bridge between C-13 and C-28 and with pronounced *anti*-leishmanial activity were isolated from the methanolic extract of leaves of the Vietnamese medicinal plant *Maesa balansae*. The structure was established on the basis of detailed NMR (COSY, NOESY, HMQC, HMBC, TOCSY and DEPT) and FAB-MS studies along with chemical degradation. All saponins identified contained the same pentaglycosidic side chain, but a different esterification pattern on the triterpenoid part. Biological evaluation of the individual compounds against visceral leishmaniasis (*Leishmania infantum* amastigotes) revealed a much better activity in vitro compared to the reference compound Pentostam®, which is currently used as first-line treatment for leishmaniasis.

1. Introduction

Maesa balansae Mez. (Myrsinaceae), a shrub growing in the Northern part of Vietnam,[1] is used in the traditional medicine for the treatment of allergies, sprains, anthelminthic infections, skin ulcers, drunkenness and headache.[2]

Previous chemical investigations on *Maesa* species demonstrated the presence of benzoquinones[3,4] and of triterpenoid compounds.[5,7–10] The present report is the first chemical investigation on *Maesa balansae*.

This paper describes the bioassay-guided isolation and structural elucidation of the main compound, a novel triterpene saponin with strong *anti*-leishmania activity, from the methanolic extract of the leaves of this medicinal plant. All of the isolated saponins possess the same pentasaccharide moiety linked to C-3 of the aglycone.

2. Results and discussion

Following a bio-assay guided screening, the dried leaves of *Maesa balansae* were extracted sequentially with dichloromethane and methanol. The residue obtained after evaporation of the methanol extract was partitioned between *n*-BuOH and water. The *n*-BuOH soluble fraction was evaporated to dryness. After stirring in acetone, the acetone insoluble fraction was repeatedly subjected to semi-preparative reversed-phase HPLC in order to obtain the pure saponins, maesabalides I (**1**), II (**2**), III (**3**), IV (**4**), V (**5**) and VI (**6**) (Fig. 1). These saponins proved to have a very pronounced *anti*-leishmanial activity (vide infra).

Maesabalide I (**1**) was obtained as a white amorphous powder. The molecular formula was established as $C_{76}H_{108}O_{32}$ on the basis of ^{13}C NMR, ^{13}C DEPT NMR and MS. The negative-ion FABMS showed an (M−H (⁻ anion at *m/z* 1531. Fragment peaks occurred at *m/z* 1385 (M−H-146(⁻, *m/z* 1239 (M−H-146-146(⁻, *m/z* 1077 (M−H-146-146-162(⁻ and *m/z* 915 (M−H-146-146-162-162(⁻, corresponding to the subsequent loss of two deoxyhexose and two hexose units.

Of the 76 carbons in the ^{13}C NMR spectrum (pyridine-D_5), 30 were assigned to the triterpenoid skeleton, 30 to the oligosaccharide moiety and the remaining 16 to two acyl groups.

Among the 30 carbons of the triterpene skeleton in the ^{13}C NMR spectrum, seven were assigned to the methyl carbons at δ 28.05, 16.62, 16.37, 18.61, 19.61, 29.80 and 20.60 ppm, and the corresponding methyl protons were identified by an HSQC experiment. Five methine carbons bearing oxygen were found at δ 89.91, 68.33, 80.80, 73.44 and 96.78 ppm.

Keywords: *Maesa balansae*; Myrsinaceae; leaves, isolation; structure elucidation; triterpene saponins; *anti*-leishmanial activity.
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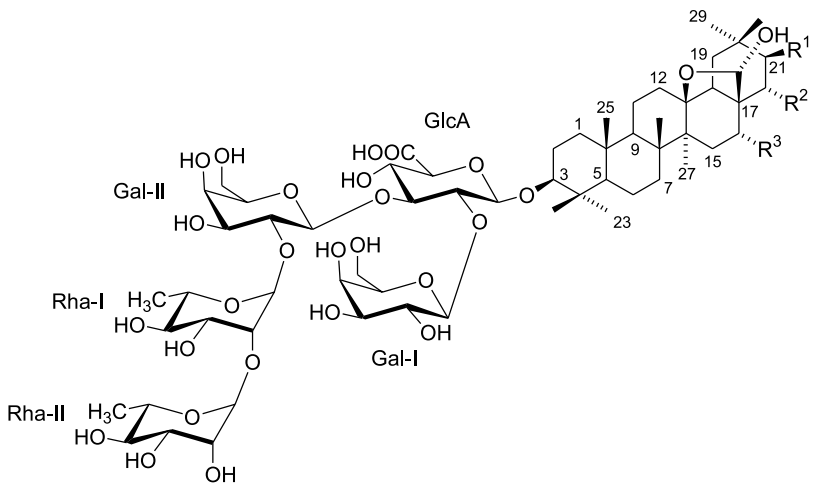

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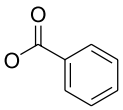
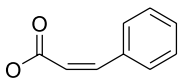
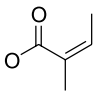
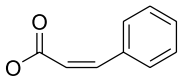
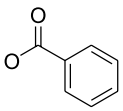
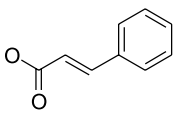
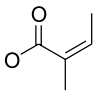
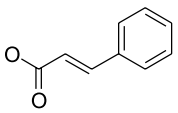
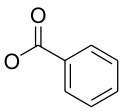
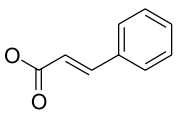
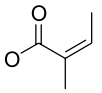
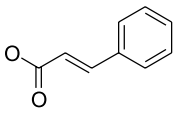
1			OH
2			OH
3			OH
4			OH
5			OAc
6			OAc

Figure 1. Saponins from the leaves of *Maesa balansae*.

The structural assignment was initiated from the long-range coupling networks observed between the methyl protons and the adjacent carbons from the HMBC experiment. Extensive NMR analysis (see Tables 1 and 2) showed that the aglycone was of an oleanane skeleton with an oxygen bridge between C-13 (δ 87.41) and C-28 (δ 96.78). This was also confirmed by comparison of the NMR data with known spectral data for structurally related compounds.[6–9] Besides the two hydroxyls at C-3 and C-28, three other hydroxyl groups were located at C-16, C-21 and C-22. The configuration was determined using the NOE information from a phase-sensitive NOESY experiment. The spatial proximity observed between H-3 and H-23, H-3 and H-5, H-16 and H-26, H-16 and H-28 indicated the β-orientation of the hydroxyl at C-3 and the α-orientation at C-16. The NOEs observed between H-22 and H-18, H-22 and H-30, between H-21 and H-29, between H-28 and H-26 and between H-28 and H-16 indicated the α-orientation of the hydroxyl group at C-22 and C-28, and the β-orientation of the hydroxyl group at C-21.

The two acyl groups, mapped out from COSY and HSQC correlations, were identified as (*Z*)-cinnamoyl and benzoyl. The (*Z*)-cinnamoyl group was attached to C-22 as established from the long-range HMBC coupling (Fig. 2) between H-22 (δ 6.57, d, J=9.9 Hz) and C_c-1 (δ 165.42) of the acyl group, H_c-2 (δ 5.84, d, J=12.8 Hz) and H_c-3 (δ 6.71, d, J=12.8 Hz) of the cinnamoyl group and C_c-1 of the acyl group and confirmed by the lowfield signal of H-22. The *cis*-configuration of the cinnamoyl group was determined from the coupling constant (12.8 Hz) between the olefinic protons H_c-2 and H_c-3. The long-range HMBC coupling between H-21 (δ 6.96, d, J=9.9 Hz) and C_b-1 (δ 166.78) of the acyl group, H_b-3 and H_b-7 (δ 8.36, d, J=7.6 Hz) of the benzoyl group and C_b-1 of the acyl group was in agreement with benzoyl substitution at C-21

Table 1. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), and HMBC spectral data of the aglycone part of maesabalide I (**1**) in pyridine-D_5[a]

Position	^{1}H (mult; *J* Hz)	^{13}C[b]	^{1}H–^{1}H COSY	HMBC[c]
1	A: 1.51 m	39.16	H1b	H25
	B: 0.77 m		H1a	
2	A: 2.19 m	26.59	H2b, H3	
	B: 1.80 m		H2a, H3	
3	3.27 brd *J*=10.8 Hz	89.91	H2a, H2b	H23, H24
4		39.86		H23, H24
5	0.73 d *J*=9.6 Hz	55.65	H6a, H6b	H23, H24, H25
6	A: 1.39 m	17.99	H5	H7
	B: 1.55 m		H5	
7	A: 1.60 m	34.38		H26
	B: 1.27 m			
8		42.65		H11, H26, H27
9	1.29 m	50.23		H25, H26
10		36.80		H25
11	A: 1.72 m	19.21	H11b	
	B: 1.45 m		H11a	
12	A: 2.16 m	33.30	H12b	
	B: 1.67 m		H12a	
13		87.41		H27, H28
14		43.76		H16, H26, H27
15	A: 2.12 m	36.39	H15b, H16	H27
	B: 1.63 m		H15a	
16	4.61 m	68.33	H15a	H22, H28
17		54.53		H22, H28
18	2.46 brd *J*=14.3 Hz	46.35	H19a	H28
19	A: 3.18 m	38.12	H19b, H18	
	B: 1.57 m		H19a	
20		37.88		H29, H30
21	6.96 d *J*=9.9 Hz	80.80	H22	H22, H29, H30
22	6.57 d *J*=9.9 Hz	73.45	H21	H21
23	1.29s	28.05		H24
24	1.20s	16.61		H23
25	0.84s	16.37		H26
26	1.35s	18.61		
27	1.65s	19.61		
28	5.20s	96.78		
29	1.18s	29.80		H30
30	1.35s	20.60		H29
Benzoyl				
1b		166.78		H21, H3b, H4b, H6b,H7b
2b		131.35		H4b, H6b
3b	8.36 d *J*=7.6 Hz	130.23	H4b	H7b
4b	7.43 m	128.84	H3b, H5b	H6b
5b	7.52 m	133.16	H4b, H6b	H3b, H7b
6b	7.43 m	128.84	H5b, H7b	H4b
7b	8.36 d *J*=7.6 Hz	130.23	H6b	H3b
Cinnamoyl				
1c		165.42		H22, H2c, H3c
2c	5.84 d *J*=12.8 Hz	120.19	H3c	
3c	6.71 d *J*=12.8 Hz	142.55	H2c	H22, H2c, H5c, H9c
4c		135.20		H2c, H6c, H8c
5c	7.52 d *J*=7.4 Hz	130.29	H6c	H2c, H3c, H6c, H7c, H8c, H9c
6c	7.18 m	128.56	H5c, H7c	H8c
7c	7.25 m	129.11	H6c, H8c	H5c, H9c
8c	7.18 m	128.56	H7c, H9c	H6c
9c	7.52 d *J*=7.4 Hz	130.29	H8c	H2c, H3c, H5c, H6c, H7c, H8c

[a] Chemical shift values are in parts per million relative to TMS. Spectra were recorded at room temperature.
[b] ^{13}C NMR multiplicities were obtained by attached proton test (APT) sequences.
[c] Protons correlated to carbon resonances in the ^{13}C column.

(Fig. 2). This fact was also confirmed by the lowfield signal of H-21.

Structural information about the basic aglycone came also from the spectral data of the aglycone **7** obtained from acid hydrolysis (Scheme 1), in which the original 13β,28 ether bridge in **1** was broken, leading to the formation of a double bond between C-12 (δ_c=125.51, δ_H=5.48, 1H, t, *J*=3.8 Hz) and C-13 (δ_c=140.56) and an aldehyde group at C-28 (δ_c=201.48, δ_H=9.24).

Moreover, the presence of five sugar moieties was evidenced by the ^{1}H and ^{13}C NMR spectra which displayed five anomeric protons at δ 4.98 (d, *J*=5.56 Hz), 5.73 (d, *J*=6.8 Hz), 6.01 (s), 6.12 (d, *J*=7.07 Hz) and 6.17 (s) and carbons at δ 105.27, 103.62, 103.48, 101.28 and 101.37,

Table 2. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), and HMBC spectral data of the carbohydrate part of maesabalide I (**1**) in pyridine-D_5[a]

Position	^{1}H (mult; *J* Hz)	^{13}C[b]	^{1}H–^{1}H COSY	HMBC[c]
GlcA				
1′	4.98 d *J*=5.6 Hz	105.27	H2′	H3, H2′, H5′
2′	4.73 m	79.85	H1′, H3′	H1″, H3′
3′	4.75 m	82.97	H2′, H4′	H1‴, H2′, H5′
4′	4.67 m	71.14		H2′, H3′, H4′
5′	4.58 m	77.11		
6′		172.50		H5′
Gal-I				
1″	5.73 d *J*=6.8 Hz	103.62	H2″	H2″
2″	4.52 m	73.45	H1″, H3″	
3″	4.29 m	75.15		H4″
4″	4.44 m	70.16		
5″	4.39 m	76.81		
6″	a: 4.53 m	62.79		H5″
	b: 4.34 m			
Gal-II		101.28		
1‴	6.12 d *J*=7.1 Hz	77.00	H2‴	
2‴	4.66 m	75.84	H1‴, H3‴	H1⁗
3‴	4.49 m	71.14		H2‴
4‴	4.47 m	76.92		H6‴
5‴	4.24 m	61.98		
6‴	4.33 m			H5‴
Rha-I		101.37		
1⁗	6.17 brs	78.01	H2⁗	H2‴
2⁗	4.87 m	72.64	H⁗, H3⁗	H1⁗
3⁗	4.75 m	74.14	H2⁗, H4⁗	H4⁗
4⁗	4.15 t *J*=9.0 Hz	69.70	H3⁗, H5⁗	H2⁗ H3⁗, H5⁗, H6⁗
5⁗	4.84 m	18.22	H4⁗, H6⁗	H1⁗, H4⁗
6⁗	1.43 d *J*=5.3 Hz		H5⁗	H4⁗
Rha-II		103.48		
1⁗′	6.01 brs	72.14	H2⁗′	H2⁗′
2⁗′	4.77 m	72.64	H1⁗′, H3⁗′	
3⁗′	4.54 m	74.14	H2⁗′, H4⁗′	H4⁗′
4⁗′	4.25 t *J*=8.8 Hz	70.16	H3⁗′, H5⁗′	H2⁗′, H6⁗′
5⁗′	4.56 m	18.48	H4⁗′, H6⁗′	H1⁗′, H4⁗′
6⁗′	1.67 d *J*=5.7 Hz		H5⁗′	H4⁗′

[a] Chemical shift values are in parts per million relative to TMS. Spectra were recorded at room temperature.
[b] ^{13}C NMR multiplicities were obtained by attached proton test (APT) sequences.
[c] [c]Protons correlated to carbon resonances in the ^{13}C column.

respectively. The low field chemical shift of C-3 (δ 79.32 for **7** to 89.91 for **1**) indicated that the pentasaccharide chain was connected to this position. This observation was confirmed by the long-range correlation between the anomeric carbon of glucuronic acid and H-3. The complete sequence of pentasaccharide side chain was determined by a combination of DQFCOSY, TOCSY, DEPT, HSQC and HMBC. Starting from the anomeric protons of each sugar unit, all the hydrogens within each spin system were assigned by DQFCOSY and TOCSY. On the basis of the

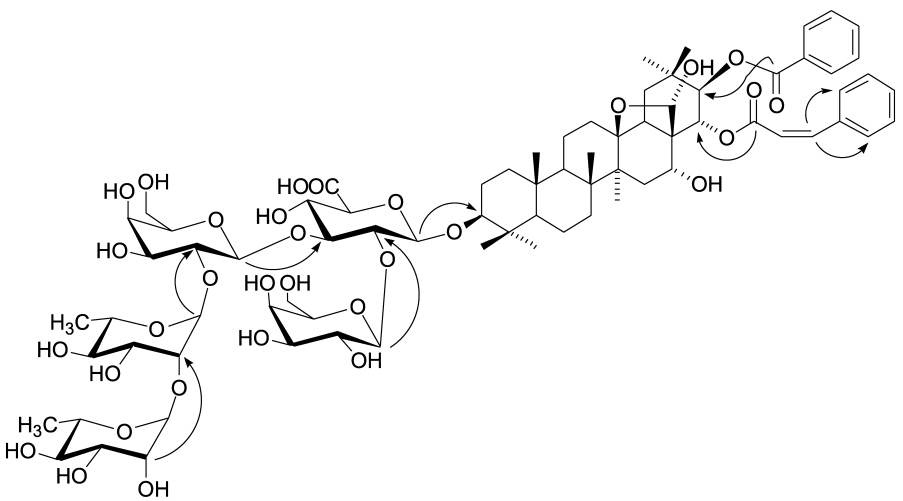
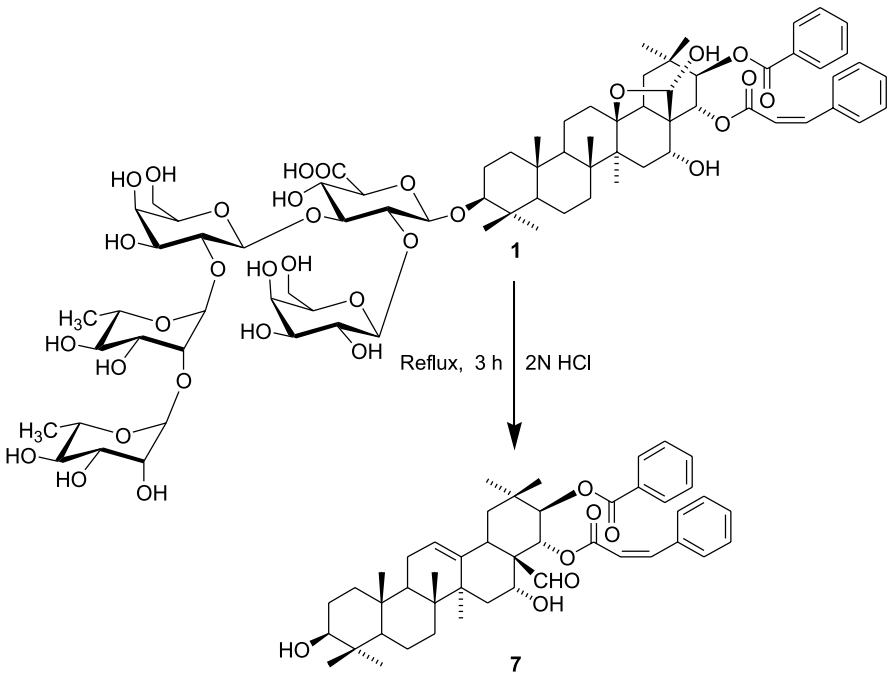


Figure 2. Characteristic long-range ^{13}C–^{1}H correlations observed in a HMBC experiment for maesabalide I (**1**) (pyridine-D_5).



Scheme 1.

assigned protons, the ^{13}C NMR resonances of each sugar unit were identified by HSQC and further confirmed by HMBC. HPTLC and HPLC of the acid hydrolysate of **1** in comparison with reference sugars, mass spectral data, NMR data of related structures[9,10] and applying the rule of Klyne[11] lead to the identification of the five monosaccharide units as β-D-glucuronic acid, β-D-galactose (×2) and α-L-rhamnose (×2). The inter-sugar linkages were established from the following HMBC correlations: H-1 of the terminal rhamnose with C-2 of rhamnose, H-1 of rhamnose with C-2 of galactose, H-1 of galactose with C-3 of glucuronic acid and H-1 of the terminal galactose with C-2 of glucuronic acid. The sugar sequence was also supported from the fragmentation patterns observed in the negative-ion FAB-MS spectra.

The β-anomeric configurations for the galactose and glucuronic acid were based on their $J_{H1,H2}$ coupling constants (5.5–8 Hz). The ^1H NMR nonsplitting pattern and the three-bond strong HMBC correlations from the anomeric proton to C-3 and C-5 (the dihedral angles between H-1 and C-3, H-1 and C-5 are about 180°), indicating the anomeric proton was equatorial, thus possessed an α-configuration. Further evidence for the α-configuration of the rhamnose residues came from the chemical shift of rhamnose C-3 (δ=72.64) and rhamnose C-5 (δ=69.70 and 70.16) in the ^{13}C NMR spectrum in comparison with ^{13}C NMR chemical shift data of the reference compounds β-L-rhamnopyranoside (C-3, δ=75.4; C-5, δ=73.5) and α-L-rhamnopyranoside (C-3, δ=72.5; C-5, δ=69.4).[12] Based upon the above evidence, maesabalide I (**1**) is established as 3-β-*O*-{[(α-L-rhamnopyranosyl (1→2)-α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→3)]-[β-D-galactopyranosyl (1→2)]-β-D-glucuronopyranosyl}-21β-benzoyloxy-22α-(*Z*)-cinnamoyloxy-13β,28-oxidoolean-16α, 28α-diol (**1**).

Maesabalide II (**2**), an amorphous solid, had a molecular formula of $C_{74}H_{110}O_{32}$, determined from the negative FAB-MS (*m/z* 1509) and ^{13}C DEPT NMR data. ^1H and ^{13}C NMR spectra (see Tables 3–6) indicated that compound **2** had the same sugar arrangement as that of saponin **1** but differed in the acyl group linked to C-21 of the aglycone. NMR analysis established the acyl group linked to C-21 to be angeloyl ((*E*)-2-methyl-2butenoyl(. Signals characteristic for the angeloyl group occurred at δ 5.93 (br q, 1H, *J*=7.2 Hz, H-3), 2.09 (br d, 3H, *J*=7.2 Hz, H-4) and 2.02 (br s, 3H, H-5) in ^1H NMR (pyridine-D_5), and at δ 167.83 (C-1), 128.87 (C-2), 137.53 (C-3), 15.94 (C-4) and 21.02 (C-5) in ^{13}C NMR (pyridine-D_5). The site of esterification was evident from the long-range HMBC correlation between the carbonyl group of angelic acid and H-21 and the lowfield signal of H-21. The structure of saponin **2** was established as 3-β-*O*-{[(α-L-rhamnopyranosyl (1→2)-α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→3)]-[β-D-galactopyranosyl (1→2)]-β-D-glucuronopyranosyl}-21β-angeloyloxy-22α-(*Z*)-cinnamoyloxy-13β,28-oxidoolean-16α, 28α-diol.

Maesabalide III (**3**) was obtained as a white amorphous powder. The negative-ion FABMS showed a (M−H (⁻ anion at *m/z* 1531. The molecular formula was established as $C_{76}H_{108}O_{32}$ on the basis of ^{13}C DEPT NMR and MS data. The ^1H and ^{13}C NMR spectra (see Tables 3–6) indicated that saponin **3** had the same sugar chain and aglycone moiety as **1**, but differed only in the (*E*)-cinnamoyl group linked to C-22 of the E-ring instead of the (*Z*)-cinnamoyl group in **1**. The *trans*-configuration was determined from the coupling constant (16.0 Hz) between the olefinic protons and the lowfield shift of the olefinic protons δ 6.39 (d, 1H, *J*=16.0 Hz, H_c-2) and δ 7.67 (d, 1H, *J*=16.0 Hz, H_c-3) in the ^1H NMR spectrum. From the above evidence the structure of **3** was elucidated as

Table 3. ^{1}H NMR data (400 MHz, *J* values in hertz) for the aglycone part of maesabalides I–VI (**1**–**6**) (pyridine-D_5) and for the semi-synthetic aglycone (**7**) ($CDCl_3$)

H	1	2	3	4	5	6	7
1	a: 1.51 m	a: 1.49 m	a: 1.52 m	a: 1.50 m	a: 1.48 m	a: 1.44 m	1.64 m
	b: 0.77 m	b: 0.79 m	b: 0.79 m	b: 0.79 m	b: 0.73 m	b: 0.74 m	0.99 m
2	a: 2.19 m	a: 2.16 m	a: 2.19 m	a: 2.16 m	a: 2.14 m	a: 2.13 m	1.60 m
	b: 1.80 m	b: 1.77 m	b: 1.80 m	b: 1.78 m	b: 1.75 m	b: 1.74 m	
3	3.27 brd *J*=10.8 Hz	3.24 brd *J*=11.6 Hz	3.26 brd *J*=12.4 Hz	3.24 brd *J*=11.2 Hz	3.19 brd *J*=11.1 Hz	3.18 brd *J*=11.4 Hz	3.23 brd *J*=11.0 Hz
4							
5	0.73 d *J*=9.6 Hz	0.71 d *J*=11.0 Hz	0.73 d *J*=11.2 Hz	0.71 d *J*=11.7 Hz	0.66 d *J*=8.4 Hz	0.65 d *J*=9.2 Hz	0.72 d *J*=11.8 Hz
6	a: 1.39 m	a: 1.39 m	a: 1.38 m	a: 1.34 m	a: 1.30 m	a: 1.30 m	1.33 m
	b: 1.55 m	b: 1.53 m	b: 1.53 m	b: 1.52 m	b: 1.52 m	b: 1.48 m	1.56 m
7	a: 1.60 m	a: 1.59 m	a: 1.60 m	a: 1.54 m	a: 1.32 m	a: 1.31 m	1.52 m
	b: 1.27 m	b: 1.23 m	b: 1.27 m	b: 1.28 m	b: 1.08 m	b: 1.05 m	1.30 m
8							
9	1.29 m	1.25 m	1.30 m	1.28 m	1.18 m	1.22 m	1.57 m
10							
11	a: 1.72 m	a: 1.71 m	a: 1.75 m	a: 1.73 m	a: 1.63 m	a: 1.73 m	1.89 m
	b: 1.45 m	b: 1.42 m	b: 1.48 m	b: 1.43 m	b: 1.41 m	b: 1.44 m	
12	a: 2.16 m	a: 2.12 m	a: 2.17 m	a: 2.14 m	a: 2.09 m	a: 2.05 m	5.48 t *J*=3.8 Hz
	b: 1.67 m	b: 1.61 m	b: 1.70 m	b: 1.64 m	b: 1.63 m	b: 1.57 m	
13							
14							
15	a: 2.12 m	a: 2.14 m	a: 2.23 m	a: 2.20 m	a: 2.23 m	a: 2.20 m	1.87 m
	b: 1.63 m	b: 1.61 m	b: 1.70 m	b: 1.68 m	b: 1.49 m	b: 1.48 m	1.37 m
16	4.61 m	4.57 m	4.83 m	4.77 m	5.97 m	5.92 m	4.50 m
17							
18	2.46 brd *J*=14.3 Hz	2.38 brd *J*=14.7 Hz	2.49 brd *J*=14.5 Hz	2.42 brd *J*=16.0 Hz	2.52 brd *J*=14.0 Hz	2.45 brd *J*=14.4 Hz	2.85 brd *J*=14.1 Hz
19	a: 3.18 m	a: 3.12 m	a: 3.20 m	a: 3.14 m	a: 2.80 m	a: 2.67 m	2.67 m
	b: 1.57 m	b: 1.51 m	b: 1.60 m	b: 1.55 m	b: 1.65 m	b: 1.60 m	1.36 m
20							
21	6.96 d *J*=9.9 Hz	6.79 d *J*=10.1 Hz	7.04 d *J*=9.8 Hz	6.86 d *J*=10.0 Hz	6.24 d *J*=10.1 Hz	6.07 d *J*=10.2 Hz	5.90 d *J*=10.1 Hz
22	6.57 d *J*=9.9 Hz	6.41 d *J*=10.1 Hz	6.60 d *J*=9.8 Hz	6.43 d *J*=10.0 Hz	6.58 d *J*=10.1 Hz	6.42 d *J*=10.2 Hz	5.51 d *J*=10.1 Hz
23	1.29s	1.27s	1.29s	1.27s	1.26s	1.25s	1.00s
24	1.20s	1.17s	1.18s	1.16s	1.16s	1.15s	0.78s
25	0.84s	0.82s	0.83s	0.81s	0.78s	0.77s	0.90s
26	1.35s	1.33s	1.36s	1.34s	1.26s	1.25s	0.66s
27	1.65s	1.61s	1.67s	1.64s	1.38s	1.35s	1.41s
28	5.20s	5.16s	5.26s	5.21s	5.32s	5.29s	9.24s
29	1.18s	1.15s	1.21s	1.18s	1.16s	1.15s	0.98s
30	1.35s	1.27s	1.37s	1.28s	1.34s	1.25s	1.21s
Acyl (C-16)							
1							
2					2.66s	2.61s	
Acyl (C-21)							
1							
2							
3	8.36 d *J*=7.6 Hz	5.93 q, overlap	8.36 d *J*=7.2 Hz	5.93 q *J*=7.3 Hz	8.30 d *J*=7.7 Hz	5.92 q *J*=7.3 Hz	8.01 d *J*=7.0 Hz
4	7.43 m	2.09 d *J*=7.2 Hz	7.40 m	2.09 d *J*=7.3 Hz	7.40 m	1.98 d *J*=7.3 Hz	7.39 m
5	7.52 m	2.02s	7.45 m	2.02s	7.47 m	1.99s	7.52 m
6	7.43 m		7.40 m		7.40 m		7.39 m
7	8.36 d *J*=7.6 Hz		8.36 d *J*=7.2 Hz		8.30 d *J*=7.7 Hz		8.01 d *J*=7.0 Hz
Acyl (C-22)							
1							
2	5.84 d *J*=12.8 Hz	5.93 d *J*=12.8 Hz	6.39 d *J*=16.0 Hz	5.93 d *J*=16.0 Hz	6.50 d *J*=16.1 Hz	6.68 d *J*=16.1 Hz	5.75 d *J*=12.5 Hz
3	6.71 d *J*=12.8 Hz	6.83 d *J*=12.8 Hz	7.67 d *J*=16.0 Hz	6.83 d *J*=16.0 Hz	7.91 d *J*=16.1 Hz	8.03 d *J*=16.1 Hz	6.86 d *J*=12.5 Hz
4							
5	7.52 d *J*=7.4 Hz	7.79 d *J*=8.1 Hz	7.24 m	7.79 m	7.25 d *J*=7.0 Hz	7.32 m	7.27–7.23 m
6	7.18 m	7.32 m	7.15 m	7.32 m	7.47 m	7.61 m	7.27–7.23 m
7	7.25 m	7.32 m	7.27 m	7.32 m	7.48 m	7.32 m	7.27–7.23 m
8	7.18 m	7.32 m	7.15 m	7.32 m	7.47 m	7.61 m	7.27–7.23 m
9	7.52 d *J*=7.4 Hz	7.79 d *J*=8.1 Hz	7.24 m	7.79 m	7.25 d *J*=7.0 Hz	7.32 m	7.27–7.23 m

3-β-*O*-{[(α-L-rhamnopyranosyl (1→2)-α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→3)]-[β-D-galactopyranosyl (1→2)]-β-D-glucuronopyranosyl}-21β-benzoyloxy-22α-(*E*)-cinnamoyloxy-13β,28-oxidoolean-16α, 28α-diol.

Maesabalide IV (**4**), a white amorphous powder, had a molecular formula of $C_{74}H_{110}O_{32}$, determined from the negative-ion FAB-MS and ^{13}C DEPT data. As for saponin **3**, compound **4** was an isomer of saponin **2** and differed only in the (*E*)-cinnamoyl group linked to C-22 instead of the (*Z*)-cinnamoyl group in saponin **2** (see Tables 3–6). The *trans*-configuration was again determined from the coupling constant (16.0 Hz) between the olefinic protons and the lowfield shift of the olefinic protons δ 6.50 (d, 1H,

Table 4. ^{1}H NMR data (400 MHz, *J* values in hertz) of the carbohydrate part of maesabalides I–VI (**1**–**6**) in pyridine-D_5

H	1	2	3	4	5	6
GlcA						
1	4.98 d *J*=5.6 Hz	4.97 d *J*=5.6 Hz	4.99 d *J*=5.8 Hz	4.97 d *J*=6.7 Hz	4.95 d *J*=5.9 Hz	4.95 d *J*=5.9 Hz
2	4.73 m	4.73 m	4.74 m	4.72 m	4.75 m	4.74 m
3	4.75 m	4.74 m	4.75 m	4.74 m	4.76 m	4.75 m
4	4.67 m	4.68 m	4.71 m	4.67 m	4.70 m	4.71 m
5	4.58 m	4.57 m	4.59 m	4.57 m	4.57 m	4.60 m
6						
Gal-I						
1	5.73 d *J*=6.8 Hz	5.73 d *J*=7.4 Hz	5.73 d *J*=7.3 Hz	5.72 d *J*=7.0 Hz	5.74 d *J*=6.6 Hz	5.74 d *J*=7.3 Hz
2	4.52 m	4.52 m	4.53 m	4.50 m	4.53 m	4.50 m
3	4.29 m	4.30 m	4.31 m	4.28 m	4.30 m	4.31 m
4	4.44 m	4.41 m	4.42 m	4.40 m	4.37 m	4.39 m
5	4.39 m	4.43 m	4.43 m	4.43 m	4.40 m	4.41 m
6	a: 4.53 m b: 4.34 m	a: 4.55 m b: 4.31 m	a: 4.56 m b: 4.35 m	a: 4.53 m b: 4.31 m	a: 4.57 m b: 4.33 m	a: 4.54 m b: 4.32 m
Gal-II						
1	6.12 d *J*=7.1 Hz	6.09 d *J*=7.7 Hz	6.09 d *J*=7.3 Hz	6.09 d *J*=7.3 Hz	6.13 d *J*=7.3 Hz	6.12 d *J*=7.8 Hz
2	4.66 m	4.66 m	4.66 m	4.65 m	4.67 m	4.67 m
3	4.49 m	4.52 m	4.51 m	4.48 m	4.51 m	4.50 m
4	4.47 m	4.48 m	4.45 m	4.46 m	4.48 m	4.48 m
5	4.24 m	4.23 m	4.31 m	4.22 m	4.25 m	4.26 m
6	4.33 m	4.33 m	4.33 m	4.33 m	4.35 m	4.36 m
Rha-I						
1	6.17 brs	6.17 brs	6.17 brs	6.16 brs	6.18 brs	6.19 brs
2	4.87 m	4.87 m	4.87 m	4.86 m	4.88 m	4.88 m
3	4.75 m	4.76 m	4.76 m	4.75 m	4.76 m	4.76 m
4	4.15 t *J*=9.0 Hz	4.16 t *J*=9.2 Hz	4.15 t *J*=9.2 Hz	4.15 t *J*=9.3 Hz	4.16 t *J*=9.2 Hz	4.16 m
5	4.84 m	4.84 m	4.83 m	4.83 m	4.86 m	4.86 m
6	1.43 d *J*=5.3 Hz	1.45 d *J*=6.1 Hz	1.44 d *J*=5.5 Hz	1.44 d *J*=5.9 Hz	1.44 d *J*=5.6 Hz	1.45 m
Rha-II						
1	6.01 brs	6.02 brs	6.01 brs	6.01 brs	6.02 brs	6.02 brs
2	4.77 m	4.78 m	4.77 m	4.77 m	4.77 m	4.77 m
3	4.54 m	4.55 m	4.56 m	4.55 m	4.55 m	4.56 m
4	4.25 t *J*=8.8 Hz	4.26 t *J*=9.5 Hz	4.26 d *J*=9.9 Hz	4.25 t *J*=9.3 Hz	4.28 t *J*=8.7 Hz	4.26 m
5	4.56 m	4.55 m	4.57 m	4.53 m	4.55 m	4.53 m
6	1.67 d *J*=5.7 Hz	1.68 d *J*=6.1 Hz	1.68 d *J*=6.1 Hz	1.67 d *J*=6.1 Hz	1.68 d *J*=5.6 Hz	1.69 m

J=16.0 Hz, H_c-2) and δ 7.84 (d, 1H, *J*=16.0 Hz, H_c-3). The structure of saponin **4** was determined as 3-β-*O*-{[(α-L-rhamnopyranosyl (1→2)-α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→3)]-[β-D-galactopyranosyl (1→2)]-β-D-glucuronopyranosyl}-21β-angeloyloxy-22α-(*E*)-cinnamoyloxy-13β,28-oxidoolean-16α, 28α-diol.

Maesabalide V (**5**), an amorphous solid, had a molecular formula of $C_{78}H_{110}O_{33}$ and a molecular weight of 1574, determined from the negative-ion FAB-MS and the ^{13}C DEPT NMR data. ^{1}H and ^{13}C NMR studies (see Tables 3–6) indicated that compound **5** had the same sugar arrangement as that of saponin **1** and the same esters linked to C-21 and C-22 as compound **3** and one acetyl group linked to C-16. Evidence for this fact was found in the extra methyl group (δ_H=2.66, δ_C=22.18) and the ester signal (δ_C=170.12) and in the long-range HMBC coupling of C-1 of the acetyl to H-16. Also the downfield shift of H-16 to δ 5.97 (δ 4.61 for **1**) and C-16 to δ 71.28 (δ 68.33 for **1**) supported this conclusion. From the above evidence, the structure of saponin **5** was established as 3-β-*O*-{[(α-L-rhamnopyranosyl (1→2)-α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→3)]-[β-D-galactopyranosyl (1→2)]-β-D-glucuronopyranosyl}-16α-acetoxy -21β-benzoyloxy-22α-(*E*)-cinnamoyloxy-13β,28-oxidoolean-28α-ol.

Maesabalide VI (**6**), an amorphous solid as well, had a molecular formula of $C_{76}H_{112}O_{33}$, and a molecular weight of 1552, determined from the negative-ion FAB-MS and the ^{13}C DEPT NMR data. ^{1}H and ^{13}C NMR studies (see Tables 3–6) indicated that compound **6** had the same sugar arrangement of that of saponin **1** and the same ester functions positioned at C-21 and C-22 as compound **4** and one acetoxy group linked to C-16 as in compound **5**. The structure of **6** was established as 3-β-*O*-{[(α-L-rhamnopyranosyl (1→2)-α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→3)]-[β-D-galactopyranosyl (1→2)]-β-D-glucuronopyranosyl}-16α-acetoxy-21β-angeloyloxy-22α-(*E*)-cinnamoyloxy-13β,28-oxidoolean-28α-ol.

The isolated saponins were tested against *Leishmania infantum* amastigotes (in vitro). Compounds **3** and **4** showed the highest activity (IC_{50}: 20 ng/mL, 0.013 nM) followed by compound **2** (IC_{50}: 50 ng/mL, 0.033 nM), compound **1** (IC_{50}: 70 ng/mL, 0.046 nM), compound **6** (IC_{50}: 700 ng/mL, 0.45 nM) and compound **5** (IC_{50}: 3400 ng/mL 2.16 nM). In comparison, Pentostam® (sodium stibogluconate), which is currently used as first line drug for the treatment of leishmaniasis[13] had an IC_{50} of 6 μg/mL (8.1 nM), which is 300 times less active than compounds **3** and **4**. In conclusion, extremely potent *anti*-leishmanial

Table 5. ^{13}C NMR data (100 MHz) of the aglycone part of maesabalides I–VI (**1**–**6**) (pyridine-D_5) and for the semi-synthetic aglycone ($CDCl_3$) (**7**)

C	1	2	3	4	5	6	7
1	39.16	39.17	39.18	39.14	39.12	39.09	38.91
2	26.59	26.57	26.57	26.53	26.56	26.56	27.55
3	89.91	89.87	89.84	89.87	89.86	89.83	79.32
4	39.86	39.85	39.86	39.81	39.84	39.83	39.15
5	55.65	55.68	55.67	55.62	55.60	55.57	55.56
6	17.99	18.00	18.01	17.95	17.85	17.82	18.63
7	34.38	34.41	34.43	34.39	34.26	34.25	33.28
8	42.65	42.66	42.68	42.63	42.63	42.60	40.07
9	50.23	50.26	50.26	50.22	50.28	50.27	46.92
10	36.80	36.81	36.82	36.77	36.77	36.75	37.34
11	19.21	19.22	19.24	19.18	19.08	19.05	23.82
12	33.30	33.61	33.33	33.29	33.11	33.10	125.51
13	87.41	87.35	87.37	87.27	86.68	86.61	140.56
14	43.76	43.77	43.80	43.75	43.19	43.17	41.76
15	36.39	36.42	36.49	36.43	33.22	33.19	34.25
16	68.33	68.32	68.50	68.45	71.28	71.24	67.86
17	54.53	54.53	54.76	54.66	53.83	53.74	57.42
18	46.35	46.35	46.27	46.19	45.42	45.39	40.42
19	38.12	38.12	38.15	38.09	37.96	37.92	46.52
20	37.88	37.55	37.85	37.44	37.60	37.25	36.51
21	80.80	79.42	80.84	79.39	79.97	78.59	77.67
22	73.45	73.70	73.92	74.02	72.19	72.33	70.07
23	28.05	28.10	28.10	28.03	28.00	27.99	28.50
24	16.61	16.64	16.63	16.58	16.56	16.55	16.01
25	16.37	16.39	16.39	16.34	16.33	16.32	15.78
26	18.61	18.62	18.64	18.59	18.45	18.44	17.50
27	19.61	19.56	19.61	19.53	19.68	19.62	27.27
28	96.78	96.83	96.88	96.87	96.06	96.05	201.48
29	29.80	29.78	29.82	29.73	29.77	29.74	29.50
30	20.60	20.77	20.62	20.68	20.07	20.22	20.04
Acyl (C-16)							
1					170.12	170.09	
2					22.18	22.16	
Acyl (C-21)							
1	166.78	167.83	166.91	167.96	166.93	167.90	166.75
2	131.35	128.87	131.45	128.97	130.84	128.25	130.36
3	130.23	137.53	130.15	136.94	130.16	138.13	130.14
4	128.84	15.94	128.79	15.81	128.90	15.95	128.79
5	133.16	21.02	133.09	20.94	133.41	20.92	133.39
6	128.84		128.79		128.90		128.79
7	130.23		130.15		130.16		130.14
Acyl (C-22)							
1	165.42	165.46	166.19	166.14	165.43	165.42	165.20
2	120.19	120.48	119.14	119.32	118.39	118.63	119.12
3	142.55	142.32	144.48	144.52	145.36	145.43	145.64
4	135.20	135.43	134.73	134.87	134.84	135.01	135.30
5	130.29	130.58	129.03	129.13	129.15	129.27	129.76
6	128.56	128.37	128.24	128.27	128.54	128.38	128.26
7	129.11	129.35	130.41	130.47	130.56	130.61	129.19
8	128.56	128.37	128.24	128.27	128.54	128.38	128.26
9	130.29	130.58	129.03	129.13	129.15	129.27	129.76

Table 6. ^{13}C NMR data (100 MHz) of the carbohydrate part of maesabalides I–VI (**1**–**6**) in pyridine-D_5

C	1	2	3	4	5	6
GlcA						
1	105.27	105.26	105.24	105.22	105.37	105.38
2	79.85	79.86	79.87	79.81	79.84	79.85
3	82.97	83.12	83.14	83.00	83.08	83.15
4	71.14	71.20	71.12	71.13	71.16	71.12
5	77.11	77.11	77.14	77.02	77.19	77.28
6	172.50	172.59	172.44	172.47	172.50	172.31
Gal-I						
1	103.62	103.73	103.71	103.64	103.70	103.74
2	73.45	73.50	73.46	73.43	73.49	73.50
3	75.15	75.25	75.21	75.18	75.26	75.28
4	70.16	70.21	70.18	70.16	70.21	70.22
5	76.81	76.89	76.83	76.85	76.94	76.95
6	62.79	62.68	62.80	62.81	62.88	62.92
Gal-II						
1	101.28	101.43	101.40	101.36	101.42	101.42
2	77.00	77.11	77.06	77.02	77.09	77.06
3	75.84	75.89	75.87	75.82	75.88	75.91
4	71.14	71.20	71.12	71.13	71.16	71.12
5	76.92	76.89	76.92	76.85	76.94	77.00
6	61.98	61.94	61.93	61.92	61.97	61.94
Rha-I						
1	101.37	101.43	101.40	101.30	101.42	101.42
2	78.01	78.01	77.99	77.99	78.06	78.07
3	72.64	72.54	72.51	72.47	72.51	72.52
4	74.14	74.13	74.09	74.08	74.12	74.14
5	69.70	69.75	69.72	69.68	69.74	69.75
6	18.22	18.29	18.26	18.21	18.28	18.28
Rha-II						
1	103.48	103.54	103.50	103.48	103.54	103.56
2	72.14	72.21	72.17	72.14	72.19	72.21
3	72.64	72.70	72.67	72.64	72.69	72.71
4	74.14	74.21	74.17	74.14	74.19	74.21
5	70.16	70.21	70.18	70.16	70.21	70.22
6	18.48	18.53	18.50	18.46	18.52	18.52

saponins were isolated from the Vietnamese medicinal plant *Maesa balansae*. These compounds are now studied further in view of their high physiological activity and potential to be developed as drug.

3. Experimental

3.1. General experimental procedures

Preparative HPLC was performed on C18 BDS (Hypersil BDS, 8 μm, 200 g) using a column with axial compression (50 mm i.d., packed at 60 bars), gradient elution: water (0.5% m/v NH_4OAc)–methanol–acetonitrile (60:20:20) to (00:50:50) in 50 min; flow rate: 80 mL/min, UV detection (275 nm). TLC on the sugar fraction was carried out on silica HPTLC-plates (Merck, Si 50000 F_{254} s, 0.2 mm layer thickness, 10×10 cm) referenced towards standard monosaccharides. The eluent consisted of $CHCl_3$–MeOH–H_2O (6.4:4.0:0.8). After spraying with 1-naphthol/H_2SO_4 reagent and heating at 110 °C for about 10 min, the spots were visualised.

^1H, ^{13}C and 2D NMR spectra were recorded using a Bruker AVANCE-400 spectrometer. The NMR data of the saponins were measured in pyridine-D_5 and the NMR data for the semi-synthetic aglycone was recorded in $CDCl_3$. Chemical shifts were expressed in δ (ppm) referring to TMS. The negative-ion mode FAB-MS spectra (for the saponins) were recorded on a Micromass VG70SEQ instrument, with glycerol as liquid matrix. The positive-ion ES-MS spectra (for the aglycone) were recorded on a micromass ZMD spectrometer coupled to an Alliance (Waters) HPLC system. Optical rotations were determined on an AA-10 automatic polarimeter (Optical Active Ltd).

3.2. Plant material

Leaves of *Maesa balansae* were collected from Deo Khe,

Dai Tu district, Thai Nguyen province in Vietnam, and were identified by Dr Tran Ngoc Ninh (Institute of Ecology and Biological Resources, NCST, Hanoi, Vietnam). Voucher specimens are deposited at the herbarium of that institute.

3.3. Extraction and isolation

Dried leaves of *Maesa balansae* (3 kg) were extracted exhaustively with dichloromethane and subsequently with methanol. After evaporation, the methanol extract was partitioned between *n*-BuOH and water. The *n*-BuOH soluble fraction was evaporated to dryness. After stirring in acetone, the acetone insoluble fraction was dried to give the crude saponin mixture (100 g). Repeated RP-18 chromatography on 10 g saponin mixture, eluted with 0.5% w/v NH_4OAc in water–methanol–acetonitrile from 60:20:20 to 0:50:50 in 50 min afforded 8 fractions. Further semi-preparative HPLC on the same column using optimised chromatographic conditions afforded six chromatographically pure saponins: maesabalide I (**1**) (230 mg), maesabalide II (**2**) (110 mg), maesabalide III (**3**) (1000 mg), maesabalide IV (**4**) (1000 mg), maesabalide V (**5**) (220 mg) and maesabalide VI (**6**) (230 mg).

3.3.1. Maesabalide I (1). White amorphous powder; FABMS (negative ion mode) *m/z*: 1531 (M−H(⁻; IR (KBr): ν_{max} 3436, 2918, 1725, 1632, 1275, 1073 cm^{-1}; $[\alpha]_D^{18}=-30.5°$ (*c*=0.53, pyridine); λ_{max} 225.0 and 274.5 nm; ^1H NMR and ^{13}C NMR are listed in Tables 1–6. Elemental analysis C: 59.08%, H: 7.33% (calculated C: 59.52%, H: 7.10%).

3.3.2. Maesabalide II (2). White amorphous powder; FABMS (negative ion mode) *m/z*: 1509 (M−H(⁻; IR (KBr): ν_{max} 3368, 2934, 1715, 1628, 1013 cm^{-1}; $[\alpha]_D^{18}=-44.4°$ (*c*=0.59, pyridine); λ_{max} 222.7 and 273.3 nm; ^1H NMR and ^{13}C NMR are listed in Tables 3–6. Elemental analysis C: 58.09%, H: 7.53% (calculated C: 58.80%, H: 7.33%).

3.3.3. Maesabalide III (3). White amorphous powder; FABMS (negative ion mode) *m/z*: 1531 (M−H(⁻; IR (KBr): ν_{max} 3414, 2934, 1719, 1634, 1285, 1074 cm^{-1}; $[\alpha]_D^{18}=-50.4°$ (*c*=0.58, pyridine); λ_{max} 221.5 and 279.2 nm; ^1H NMR and ^{13}C NMR are listed in Tables 3–6. Elemental analysis C: 59.25%, H: 7.24% (calculated C: 59.52%, H: 7.10%).

3.3.4. Maesabalide IV (4). White amorphous powder; FABMS (negative ion mode) *m/z*: 1509 (M−H(⁻; IR (KBr): ν_{max} 3415, 2934, 1705, 1635, 1282, 1079 cm^{-1}; $[\alpha]_D^{18}=-45.3°$ (*c*=0.75, pyridine); λ_{max} 216.8 and 279.2 nm; ^1H NMR and ^{13}C NMR are listed in Tables 3–6. Elemental analysis C: 59.19%, H: 7.24% (calculated C: 58.80%, H: 7.33%).

3.3.5. Maesabalide V (5). White amorphous powder; FABMS (negative ion mode) *m/z*: 1573 (M−H(⁻; IR (KBr): ν_{max} 3438, 2927, 1723, 1636, 1275, 1075 cm^{-1}; $[\alpha]_D^{18}=-61.5°$ (*c*=0.59, pyridine); λ_{max} 222.7 and 278.0 nm; ^1H NMR and ^{13}C NMR are listed in Tables 3–6. Elemental analysis C: 59.42%, H: 7.18% (calculated C: 59.64%, H: 7.04%).

3.3.6. Maesabalide VI (6). White amorphous powder; FABMS (negative ion mode) *m/z*: 1551 (M−H(⁻; IR (KBr): ν_{max} 3385, 2935, 1719, 1635, 1268, 1044 cm^{-1}; $[\alpha]_D^{18}=-54.8°$ (*c*=0.68, pyridine); λ_{max} 221.5 and 278.0 nm; ^1H NMR and ^{13}C NMR are listed in Tables 3–6. Elemental analysis C: 59.03%, H: 7.12% (calculated C: 58.75%, H: 7.27%).

3.4. Acid hydrolysis

Compound **1** (100 mg) was dissolved in 10 mL 2N HCl solution (H_2O–MeOH 1:1) and the mixture was refluxed while stirring for 3 h. After evaporation of the methanol in vacuo, the solution was extracted with EtOAc (3×4 mL). The combined organic layers were washed with H_2O and then evaporated to dryness to give an amorphous powder, which was subjected to HPLC purification obtaining compound **7** (*m/z* 723 (M+H(⁺) (32 mg, 68%). Conditions: column Hypersil C18-BDS, 5 μm, 21.2×250 mm, 30 mL/min, H_2O–MeCN 20:80 to 100% MeCN in 30 min, UV detection at 275 nm. The H_2O layer was concentrated and compared with standard monosaccharides by silica gel TLC, using the mixture $CHCl_3$–MeOH–H_2O (6.4:4.0:0.8) and visualization by spraying with 1-naphthol-H_2SO_4. D-glucuronic acid, D-galactose and L-rhamnose were identified.

3.5. Analysis of the carbohydrate fraction by HPLC

The aqueous fraction obtained after acid hydrolysis of **1** was evaporated to dryness and resolubilized at 100 ppm in water. The chromatogram was compared with those of standard monosaccharides: D-glucose, D-galactose, L-rhamnose, L-arabinose, D-xylose, D-fucose, D-glucuronic acid and D-galacturonic acid injected at a concentration of 50 ppm. D-galactose (×2), D-glucuronic acid and L-rhamnose (×2) were identified after co-elution of the aqueous fraction with the corresponding standard monosaccharides. Conditions: pre-column: PA1 Guard, 10-32, column: CarboPac™ PA1, 10 μm, 250×4 mm, 1 mL/min, 25 μL loop (injection after 10 min), pulsed amperometric detector, solvent A: 100 mM NaOH, solvent B: 100 mM NaOH+0.7 M NaOAc, solvent C: 1 M NaOH, solvent D: H_2O. Linear gradient system: 0.0 min: 100% C, 2.9 min: 100% C, 3.0 min: A–D 5:95, 35.0 min: A–D 5:95, 45.0 min: A–B–D 20:20:60, 55.0 min: A–B 20:80.

3.6. Bioassays

A laboratory strain of *Leishmania infantum* (MHOM/MA(BE)/67), known to be sensitive to the available *anti*-leishmanial reference drug was used. The compounds for biological testing were prepared in 100% dimethyl sulphoxide at 4 concentrations (32–8–2–0.5 μM or μg/mL). Reference drug was sodium-stiboguconate (Pentostam®, GSK). The in vitro sensitivity of amastigotes to the test compounds was determined in primary mouse peritoneal macrophages. These macrophages were induced in mice by intraperitoneal administration of 2% potato starch and harvested about 24 h later in RPMI-1640 medium. Assays were performed in triplicate in 96-well tissue culture plates, each well containing the compound dilutions together with 3×10^4 macrophages and 3×10^5 parasites/well. After 5 days incubation at 37 °C, intracellular amastigote burdens were

microscopically assessed after Giemsa staining. The results are expressed as % reduction of parasite burden compared to untreated control wells. The IC_{50} was lower than 1 μg/mL or μM and no cytotoxicity against MRC-5 cells was observed. Therefore, the compounds were classified as highly active. A detailed account on the bioassays (in vitro and in vivo) will be reported in a medicinal chemistry journal.

References

1. Pham Hoang Ho, Cay co Vietnam (an illustrated Flora of Vietnam), *1*, NHA XUAT BAN TRE, Vietnam, 1999, 675.
2. Vo Van Chi, Tu dien cay thuoc Vietnam, NHA XUAT BAN Y HOC, Vietnam, 1997, 489.
3. Ogawa, H.; Natori, S. *Phytochemistry* **1968**, *7*, 773–782.
4. Kubo, I.; Kim, M.; Ganjian, I.; Kamikawa, T.; Yamagiwa, Y. *Tetrahedron* **1987**, *43*, 2653–2660.
5. Chakravarty, A. K.; Das, B.; Pakrashi, S. C. *Phytochemistry* **1987**, *26*, 2345–2349.
6. Doddrell, D. M.; Khong, P.; Lewis, K. G. *Tetrahedron Lett.* **1974**, *27*, 2381.
7. Sindambiwe, J. B.; Baldé, A. M.; De Bruyne, T.; Pieters, L.; Van Den Heuvel, H.; Claeys, M.; Vanden Berghe, D. A.; Vlietinck, A. J. *Phytochemistry* **1996**, *41*, 269–277.
8. Koike, K.; Kudo, M.; Jia, Z.; Nikaido, T.; Ide, Y.; Sakura, T. *J. Nat. Prod.* **1999**, *62*, 228–232.
9. Apers, S.; De Bruyne, T. E.; Claeys, M.; Vlietinck, A. J.; Pieters, L. A. C. *Phytochemistry* **1999**, *52*, 1121–1131.
10. Jiang, Z.; Gallard, J. F.; Adeline, M. T.; Dumontet, V.; Tri, M. V.; Sévenet, T.; Païs, M. *J. Nat. Prod.* **1999**, *62*, 873–876.
11. Klyne, W. *Biochem. J.* **1950**, *47*, xli–xlii.
12. Agrawal, P. K.; Jain, D. C.; Gupta, R. K.; Thakur, R. S. *Phytochemistry* **1985**, *24*, 2479–2496.
13. Olliaro, P. L.; Bryceson, A. D. M. *Parasitol. Today* **1993**, *9*, 323–328.