Notes

Flavonol and Chalcone Ester Glycosides from *Bidens leucantha*

Nunziatina De Tommasi* and Cosimo Pizza

Centro Interdipartimentale di Chimica, Biologia e Tecnologie Farmaceutiche, Università degli Studi di Salerno, Piazza Vittorio Emanuele 9, Penta di Fisciano, Salerno, Italy

Rita Aquino

Dipartimento di Chimica delle Sostanze Naturali, Università "Federico II" di Napoli, Napoli, Italy

Jativa Cumandà

Escuela Superior Politecnica del Chimborazo, Panamericana sur, Riobamba, Ecuador

Naheed Mahmood

MRC Collaborative Centre, 1−3 Burtonhole Lane, Mill Hill, London, U.K.

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Four new flavonol 7-*O*-glycosides **1**−**4** and a new chalcone ester glycoside **5** have been isolated from the leaves of *Bidens leucantha* along with four known chalcone ester glycosides **6**−**9**. Their structures were elucidated using a combination of spectroscopic techniques. The in vitro anti-HIV activity of all the compounds was tested as part of our screening of potential anti-AIDS agents from medicinal plants. Although none of the flavonol glycosides was active, a moderate inhibitory effect on viral replication was exerted by chalcone ester glycoside **9**.

In our continuing search for bioactive compounds from South American medicinal plants, we have examined *Bidens leucantha* Willd. (Asteraceae), a plant used in Ecuadorian folk medicine as an antiinflammatory agent.[1] The nine compounds isolated from the MeOH extract of the leaves comprised four new glycosides (**1**−**4**) having quercetin 3-methyl ether or quercetin 3,4′-dimethyl ether as the aglycons and an oligosaccharide portion linked at C-7 made up of two or three sugars (glucose and rhamnose). A new chalcone ester glycoside (**5**) based on 4,2′,3′,4′-tetrahydroxychalcone and four known chalcone ester glycosides (**6**−**9**) possessing okanin as the aglycon moiety were also obtained from the plant.

A Sephadex LH-20 column and reversed-phase HPLC separations of the MeOH extract of the leaves of *B. leucantha* provided compounds **1**−**9**. The molecular formulas $C_{28}H_{32}O_{16}$ for compound **1**, $C_{29}H_{34}O_{16}$ for **2,** $C_{35}H_{44}O_{20}$ for **3**, and $C_{35}H_{44}O_{21}$ for **4** were determined by negative ion FABMS and ^{13}C- and DEPT ^{13}C-NMR analysis. The fragmentation patterns suggested the loss of a deoxyhexose (146 mass units) and a hexose (162 mass units) from the quasi-molecular ions at *m*/*z* 623 and 637, respectively, in compounds **1** and **2**; the loss of a deoxyhexose and two hexose units from the quasi-molecular anion at *m*/*z* 799 in **4**; and the loss of two deoxyhexose units and a hexose from the quasi-molecular anion at *m*/*z* 783 in **3**.

The ^{1}H-NMR spectra of **1**−**4** indicated that quercetin 3-methyl ether[2,3] [δ 3.84 (3H, s, −OMe at C-3), 6.54 (1H, d, J = 2 Hz, H-6), 6.73 (1H, d, J = 2 Hz, H-8), 6.90 (1H, d, J = 8 Hz, H-5′), 7.71 (1 H, d, J = 1.5 Hz, H-2′), 7.65 (1H, dd, J = 8, 1.5 Hz, H-6′)] was the aglycon of compound **1** and that quercetin 3,4′-dimethyl ether[2,3] [δ 3.84, 3.98 (−OMe at C-4′), 6.54 (H-6), 6.75 (H-8), 7.14 (H-5′), 7.66 (H-6′), 7.71 (H-2′)] was the aglycon of **2**, **3**, and **4**. The ^{13}C-NMR chemical shifts of C-2 (δ 157.7), C-3 (δ 139.9), and C-4 (δ 180.1) (Table 1) were indicative of the 3-*O*-methyletherification.[4] In their ^{1}H-NMR spectra, the unusual 7-*O*-glycosylation was indicated by downfield shifts of H-6 (ca. +0.32 ppm) and H-8 (ca. +0.33 ppm) with respect to rutin[5] as model compound. Similarly, in the ^{13}C-NMR spectra of **1**−**4** (Table 1) the 7-*O*-glycosylation was confirmed by the diagnostic[4] upfield shift of C-7 (−2.0 ppm) and by downfield shifts of the *ortho*-related C-8 (+0.8 ppm) and C-6 (+1.2 ppm) and *para*-related C-10 (+1.6 ppm) carbons with respect to rutin. The sugar moieties were shown to be rutinose[4,5] [α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside] in **1** and **2**, 2^G-glucopyranosylrutinoside {β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside} in **4** and [α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside] in **3** by NMR data. The exact disposition of the three monosaccharide units and the position of the interglycosidic linkages in compounds **3** and **4** was achieved using 2D NMR spectroscopy. HOHAHA (2D homonuclear Haztmann-Hahn spectroscopy) experiments allowed resolution of the overlapped spectral region of the trisaccharide moiety of **3** and **4** into a subset of individual monosaccharide spectra; 2D-COSY-90 experiments established the proton sequence within each sugar fragment starting from the well-resolved

* To whom correspondence should be addressed. Phone: 39-89-968954. FAX: 39-89-968937.

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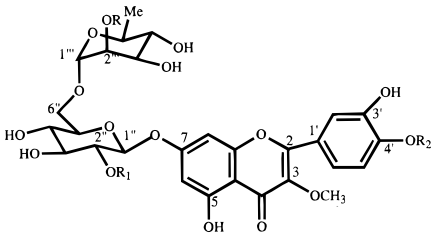
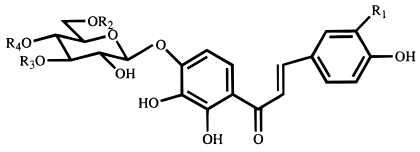
Table 1. ^{13}C-NMR Data, Aglycon Portion, of Compounds **1**, **2**, **5** in CD_3OD

	1	**2**[a]		**5**
position	δC	δC	position	δC
2	157.7	157.8	1	126.8
3	139.9	139.9	2	131.3
4	180.0	180.1	3	117.8
5	164.3	164.3	4	161.4
6	101.2	101.6	5	117.8
7	164.5	164.5	6	131.3
8	95.8	95.8	α	118.2
9	158.8	159.20	β	147.0
10	107.1	107.2	>C=O	197.6
1′	123.6	123.8	1′	117.6
2′	116.2	116.0	2′	154.3
3′	145.0	147.0	3′	136.9
4′	149.6	151.6	4′	150.8
5′	117.3	112.3	5′	109.3
6′	123.3	123.3	6′	121.1
−OMe at C-3	58.10	58.10		
−OMe at C-4′		60.2		

[a] ^{13}C-NMR data for the aglycons of compounds **3** and **4** were superimposable on those of compound **2**.



	R	R_1	R_2
1	-H	-H	-H
2	-H	-H	$-CH_3$
3	Rha-II	-H	$-CH_3$
4	-H	Glc-II	$-CH_3$



	R_1	R_2	R_3	R_4
5	-H	p-coumaroyl	-H	-H
6	-OH	p-coumaroyl	-H	Ac
7	-OH	Ac	-H	H
8	-OH	Ac	Ac	H
9	-OH	Ac	Ac	Ac

Rha= α-L-rhamnopyranosyl; Glc=β-D-glucopyranosyl

anomeric proton signals of a β-D-glucopyranose (δ 5.10, d, J = 7.5 Hz), and two α-L-rhamnopyranose units (δ 5.00 and 4.78, each d, J = 1.5 Hz) in compound **3**, as well as α-L-ramnopyranose (δ 4.80) and two β-D-glucopyranose units (δ 5.15 and 4.62) in compound **4**; HETCOR correlated all proton signals with those of each corresponding carbon leading to the assignments in Table 3. Chemical shifts, multiplicity of the proton signals, values of coupling constants, and chemical shifts of carbons indicated that the sugars must be in the β-D-glucopyranosyl and in the α-L-rhamnopyranosyl forms.

In compound **3** a glycosidation shift at C-6″ (ca. +6.0 ppm) and the chemical shifts of H-1″ (δ 5.10) and C-1″ (δ 101.2) of glucose indicated this monosaccharide to be glycosidated at C-6 and linked at the aglycon. Of the two signals due to anomeric protons of rhamnose (δ 5.00 and 4.78, each d, J = 1.5 Hz), the one at higher field (δ 4.78, H-1‴), correlating to the C-1‴ resonance at δ 102.4 by HETCOR, indicated that the rhamnose I unit was linked to a secondary alcoholic carbon (C-6″ of the glucose).[6] By HOHAHA, this proton showed connectivities with a signal δ 3.90 (dd, J = 1.5, 2.5 Hz, H-2‴ by COSY), which was correlated by HETCOR to the carbon resonance at δ 79.8 (C-2‴) showing glycosylation at position 2. Therefore, these signals were assigned to the 1,2-glycosylated rhamnose I unit. The anomeric proton at δ 5.00 (correlated to the C-1⁗ resonance at δ 104.0 by HETCOR) was assigned to the rhamnose II unit linked to a tertiary alcoholic carbon[6] (C-2‴ of rhamnose I). The rhamnose II was determined to be terminal by the absence of any glycosylation shift.[6] These deductions were confirmed by a COLOC spectrum, which showed some diagnostic long-range correlations between H-1″ of glucose (δ 5.10) and C-7 (δ 164.5) of the aglycon, between H-1‴(δ 4.78) of the rhamnose I unit and C-6″ (δ 67.4) of glucose, between H-5‴(δ 4.15) and C-3‴ (δ 71.10), and between H-1⁗ (δ 5.00) and C-2‴ (δ 79.8) (see Table 3).

The combined use of the techniques mentioned above as well as comparison with literature data showed that the trisaccharide moiety of **4** was formed by a glucose branched at C-2″ (δ 82.8) and C-6″ (δ 68.2) by an unsubstituted rhamnose and an unsubstituted glucose II unit (Table 3). The relative positions of the two sugars indicated by the chemical shift (δ 4.80) of H-1‴, typical of a rhamnose linked to a secondary alcoholic carbon (C-6″ of glucose),[6] were confirmed by NOEDS experiments. By irradiation of the signal at δ 4.80 (H-1‴ of rhamnose) we observed a NOE with the signals at δ 4.02 and 3.60 (H_2-6″ of glucose). Thus, rhamnose should be linked at C-6″ and the terminal glucose II unit at C-2″.

The molecular formula $C_{30}H_{28}O_{12}$ of compound **5** was determined by FABMS and ^{13}C and DEPT ^{13}C NMR analysis. Its negative FABMS spectrum showed a quasi-molecular ion at m/z 579 and peaks at m/z 433 $[(M - H) - 146]^-$ and at m/z 271 $[(M - H) - 146 - 162]^-$ ascribable to the loss of coumaroyl and glucosyl moieties. The ^{1}H-NMR spectrum exhibited the characteristic pattern of a chalcone with a hydroxyl group at position 4 of the B-ring and a 2′,3′,4′ trioxygenated A-ring showing two *ortho*-coupled (J = 8 Hz) doublet signals, each integrating for two H, at δ 6.98 (H-2 and H-6) and 7.05 (H-3 and H-5) along with two *ortho*-coupled H signals at δ 6.80 and 7.55 (each d, J = 8 Hz, H-5′ and H-6′). The doublet (J = 7.5 Hz) signal at δ 5.05 was assignable to the anomeric proton of a β-D-glucopyranose. Two signals at δ 4.25 and 4.64 appeared as the AB portion of an ABX system (J_{AB} = 12.5, J_{AX} = 2.5, J_{BX} = 4.5 Hz) and indicated the C-6″ esterification of the glucose. These results were consistent with the typical downfield shift of the C-6″ signal (+1.7 ppm) and the upfield shift (−1.3 ppm) of C-5″ of glucose moiety in the ^{13}C-NMR spectrum of compound **5** (Table 2) with

Table 2. ^{13}C-NMR Data of Sugar Moieties of Compounds **1**, **2**, and **5** (CD_3OD)

position	**1**	**2**	position	5
Glc[a] 1″	101.3	101.0	Glc 1″	99.6
2″	73.8	74.0	2″	73.7
3″	77.6	77.9	3″	76.9
4″	71.1	71.4	4″	71.8
5″	77.2	77.4	5″	76.6
6″	67.3	68.2	6″	64.4
Rha[b] 1‴	101.9	102.2	*p*-coumaroyl 1‴	124.9
2‴	72.2	72.1	2‴	130.2
3‴	71.8	72.4	3‴	116.5
4‴	74.5	74.0	4‴	161.0
5‴	69.6	69.7	5‴	116.5
6‴	17.6	17.8	6‴	130.2
			7‴	146.8
			8‴	118.0
			9‴	168.7

[a] Glc = β-D-glucopyranosyl. [b] Rha = α-L-rhamnopyranosyl.

respect to homologous carbons of unsubstituted glucose.[7−9] The ester group linked at C-6″ was a *trans-p*-coumaroyl residue as deduced by ^{1}H [δ 6.38 (1H, d, J = 16 Hz, H-8‴), 7.45 (2H, d, J = 8 Hz, H-2‴ and H-6‴), 6.73 (2H, d, J = 8 Hz, H-3‴ and H-5‴), 7.41 (1H, d, J = 16 Hz, H-7‴)] and ^{13}C-NMR spectra in agreement with published data.[7−9] The 4′-*O*-glycosylation was deduced by superimposition of the A-ring signals with those of okanin-4′-*O*-glucoside[7−9] and its known ester derivatives **6**−**9.** The structure of compounds **6**−**9** were unequivocal from their spectral data by comparison with literature data.[7−9]

In our continuing search for potential anti-HIV agents from natural sources a number of flavans, flavones, and flavanones[10] have been tested, while no data are available on chalcone ester glycosides. Therefore, the anti-HIV activity and toxicity of compounds isolated from *B. leucantha* were tested in C8166 cells infected with HIV-1_{MN}. The inhibition of HIV infection in vitro was determined as previously reported.[10−12] Although none of the flavonol glycosides **1**−**4** was found to be effective, moderate antiviral activity was observed for chalcone ester derivatives **5**, **6**, and **9**. Compound **9** showed an EC_{50} value of 20 μg/mL, no cytotoxicity at 250 μg/mL, and the selectivity index was over 12.5. Compound **5** elicited lower potency (EC_{50} = 50 μg/mL, TC_{50} = 250 μg/mL, S. I. = 5), and compound **6** was comparable to compound **9**, showing a selectivity index of over 10 (EC_{50} = 10 μg/mL, TC_{50} over 100 μg/mL).

Experimental Section

General Experimental Procedures. A Bruker AMX-500 spectrometer equipped with a Bruker X-32 computer using the UXNMR software package was used for NMR measurements in CD_3OD solutions. 2D homonuclear proton chemical shift correlation (COSY), 2D HOHAHA, ^{1}H−^{13}C HETCOR, and COLOC experiments were performed as described previously.[13] NOE experiments were performed using the spectral subtraction technique (NOEDS). The sample for NOE measurements was previously degassed by bubbling argon though the solution for 40 min. Optical rotations were measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm in 1% w/v solutions in MeOH. FABMS were recorded in a glycerol matrix in the negative ion mode on a VG ZAB instrument (XE atoms of energy of 2−6 kV). HPLC separations were performed with a Waters model 6000A pump equipped with a U6K injector and a model 401 refractive index detector.

Plant Material. *B. leucantha* Willd. was collected in Ecuador in June 1993, and identified by W. Palacios.

Table 3. NMR Data of the Sugar Moieties of Compounds **3** and **4** in CD_3OD

	3			4	
sugar	δC[a]	δH[b,e]	COLOC[c]	δC[a]	δH[b,e]
Glc I					
1	101.2	5.10 d, J = 7.5	164.5	100.0	5.15 d, J = 7.0
2	73.9	3.5 dd, J = 7.5, 9.0		82.8	3.52 dd, J = 7.0, 9.0
3	78.2	3.42 t, J = 9.0		77.9	3.42 t, J = 9.0
4	71.4	3.45 t, J = 9.0		71.4	3.34[d]
5	77.2	3.30[d] m		77.1	3.30[d] m
6	67.4	3.60 dd, J = 12.0, 3.0		68.2	3.60 dd, J = 12.0, 3.0
		4.00 dd, J = 12.0, 5.0			4.02 dd, J = 12.0, 5.0
Rha I					
1	102.4	4.78 d, J = 1.5	67.4	102.2	4.80 d, J = 1.5
2	79.8	3.90 dd, J = 1.5, 2.5		72.1	3.94 dd, J = 1.5, 3.4
3	71.1	3.70 dd, J = 2.5, 9.5		72.4	3.88 dd, J = 3.4, 9.5
4	75.4	3.45 t, J = 9.5		74.0	3.55 t, J = 9.5
5	68.6	4.15 dq, J = 9.5, 6.5	71.10	69.7	4.20 dq, J = 9.5, 6.5
6	18.4	1.28 d, J = 6.5		17.8	1.12 d, J = 6.5
Rha II					
1	104.0	5.00 d, J = 1.5	79.8		
2	73.8	3.92 dd, J = 1.5, 3.4			
3	72.2	3.79 dd, J = 3.4, 9.5			
4	75.1	3.55 t, J = 9.5			
5	69.7	4.12 dq, J = 9.5, 6.5			
6	17.9	1.30 d, J = 6.5			
Glc II					
1				104.0	4.62 d, J = 7.3
2				75.5	3.32 dd, J = 7.3, 9.0
3				77.9	3.34[d]
4				71.4	3.36 t, J = 9.0
5				78.2	3.38 m
6				62.6	3.69 dd, J = 12.0, 3.0
					3.82 dd, J = 12.0, 5.0

[a] Assignments confirmed by HETCOR experiments. [b] Assignments confirmed by combination of COSY and HOHAHA results. [c] Selected connectivities observed across the glycosidic linkages. [d] Overlapping signals. [e] The coupling constants are given in Hz.

A voucher specimen is deposited in the herbarium of Escuela Superior Politecnica del Chimborazo, Riobamba, Ecuador.

Extraction and Isolation. The air dried, powdered leaves (498 g) of *Bidens leucantha* were defatted with petroleum ether and $CHCl_3$ and then extracted with MeOH (18.5 g). Concentration and extraction with H_2O−*n*-BuOH gave an organic fraction (8 g). Chromatography of part of this fraction (4 g) on a Sephadex LH-20 column eluting with MeOH (100 × 5 cm) gave fractions (8 mL) checked by TLC [SiO_2 plates, *n*-BuOH−AcOH−H_2O (12:3:5) and $CHCl_3$−MeOH−H_2O (40:9:1)] and combined in three main fractions. Fraction A from Sephadex LH-20 column was fractionated by RP−HPLC on a C18 μ-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL/min) with MeOH−H_2O (1:1) to yield compounds **1** (t_R = 15 min , 16 mg), **2** (t_R = 17 min, 20 mg), **3** (t_R = 12 min, 30 mg), and **4** (t_R = 10 min, 32.5 mg). Fraction B was separated by RP−HPLC with MeOH−H_2O (9:11) to yield compounds **5** (t_R = 8 min, 14 mg) and **6** (t_R = 11 min, 10 mg). Fraction C, using the same conditions, yielded compounds **7** (t_R = 9 min, 28 mg), **8** (t_R = 13 min, 19 mg), and **9** (t_R = 15 min, 20 mg).

Compound 1: $[\alpha]^{25}_D$ + 15.5° ; mp 204−206 °C; FABMS *m/z* (M − H)$^-$ 623, [(M − H) − 146]$^-$ 477, [(M − H) − (146 + 162)]$^-$ 315; ^{1}H NMR δ 3.84 (3H, s, −OMe at C-3), 3.92 (1H, dd, *J* = 12.0, 3.0 Hz, H-6a″), 4.07 (1H, dd, *J* = 12.0, 5.0 Hz, H-6b″), 4.74 (1H, d, *J* = 1.5 Hz, H-1‴), 5.10 (1H, d, *J* = 7.0 Hz, H-1″), 6.54 (1H, d, *J* = 2 Hz, H-6), 6.73 (1H, d, *J* = 2 Hz, H-8), 6.90 (1H, d, *J* = 8 Hz, H-5′), 7.65 (1H, dd, *J* = 8, 1.5 Hz, H-6′), 7.71 (1 H, d, *J* = 1.5 Hz, H-2′). For ^{13}C NMR, see Tables 1 and 2.

Compound 2: $[\alpha]^{25}_D$ +18°; mp = 196−198 °C; FABMS *m/z* (M − H)$^-$ 637, [(M − H) − 146]$^-$ 491, [(M − H) − (146 + 162)]$^-$ 329; ^{1}H NMR δ 3.84 (3H, s, −OMe at C-3), 3.98 (3H, s, −OMe at C-4′), 3.90 (1H, dd, *J* = 12.0, 3.0 Hz, H-6a″), 4.05 (1H, dd, *J* = 12.0, 5.0 Hz, H-6b″), 4.75 (1H, d, *J* = 1.5 Hz), H-1‴), 5.12 (1H, d, *J* = 7.0 Hz, H-1″), 6.54 (1H, d, *J* = 2.0 Hz, H-6), 6.75 (1H, d, *J* = 2.0 Hz, H-8), 7.14 (1H, d, *J* = 8.0 Hz, H-5′), 7.66 (1H, dd, *J* = 8, 1.5 Hz, H-6′), 7.71 (1H, d, *J* = 1.5 Hz, H-2′). For ^{13}C NMR, see Tables 1 and 2.

Compound 3: $[\alpha]^{25}_D$ +8.5°; mp 205−207 °C; FABMS *m/z* (M − H)$^-$ 783, [(M − H) − 146]$^-$ 637, [(M − H) − (2 × 146)]$^-$ 491, [(M − H) − (2 × 146 + 162)]$^-$ 329; ^{1}H NMR of the aglycon δ 3.82 (3H, s, −OMe at C-3), 3.96 (3H, s, −OMe at C-4′), 6.51 (H-6), 6.76 (H-8), 7.12 (H-5′), 7.68 (H-6′), 7.71 (H-2′). For ^{13}C NMR of the aglycon, see Table 1. For NMR data of sugar moiety, see Table 3.

Compound 4: $[\alpha]^{25}_D$ +10.7°; mp 193−195 °C; FABMS *m/z* (M − H)$^-$ 799, [(M − H) − 146]$^-$ 653, [(M − H) − 162]$^-$ 637, [(M − H) − (162 + 146)]$^-$ 491, [(M − H) − (2 × 162 + 146)]$^-$ 329; ^{1}H NMR δ 3.85 (3H, s, −OMe at C-3), 3.96 (3H, s, −OMe at C-4′), 6.55 (H-6), 6.78 (H-8), 7.14 (H-5′), 7.72 (H-6′), 7.73 (H-2′). For ^{13}C NMR, see Tables 1 and 3.

Compound 5: [a]$^{25}_D$ +80.0°; mp >150 °C (dec); FABMS *m/z* (M − H)$^-$ 579, [(M − H) − 146]$^-$ 433, [(M − H) − (146 + 162)]$^-$ 271; ^{1}H NMR δ 3.40−3.60 (3H, overlapped, H-3″, H-2″, H-4″), 3.61 (1H, m, H-5″), 4.25 (1H, dd, *J* = 12.5, 2.5 Hz, H-6″a), 4.64 (1H, *J* = 12.5, 4.5, H-6b″), 5.05 (1H, d, *J* = 7.5 Hz, H-1"), 6.38 (1H, d, *J* = 16 Hz, H-8‴), 6.73 (2 H, d, *J* = 8 Hz, H-3‴ and H-5‴), 6.80 (1H, d, *J* = 8 Hz, H-5′), 6.98 (double signal, H-2 and H-6, d, *J* = 8 Hz), 7.05 (double signal, H-3 and H-5, d, *J* = 8 Hz), 7.41 (1H, d, *J* = 16 Hz, H-7‴), 7.45 (2 H, d, *J* = 8 Hz, H-2‴ and H-6‴), 7.55 (1H, d, *J* = 8 Hz, H-6′). For ^{13}C NMR, see Tables 1 and 2.

Antiviral Assay. The anti-HIV activity and toxicity of compounds isolated from *B. leucantha* were tested in C8166 cells infected with HIV-1_{MN}. Cells were cultured in RPMI 1640 with 10% fetal calf serum; 4 × 10^4 cells per microtiter plate well were mixed with five-fold dilutions of compounds **1**−**9** prior to addition of 10 $CCID_{50}$ (50% cell culture infections dose) units of virus and incubated for 5−6 days. Formation of syncitia was examined from 2 days post-infection. The inhibition of HIV infection was determined by: syncitium-forming assay, estimation of antigen gp120 by ELISA and cell viability determinations of virus-infected and uninfected cells by the XTT-formazan method.[10−12] Results are expressed as EC_{50}, which represents the concentration in μg/mL that reduces the production of Ag gp120 by 50% in infected C8166 cells. TC_{50} represents the concentration in μg/mL of drug that causes 50% of cytotoxicity to uninfected C8166 cells.

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