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Acetylated pseudoguaianolides from *Parthenium hysterophorus* and their cytotoxic activity ☆

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Abstract

Chemical examination of the flowers of *Parthenium hysterophorus* has resulted in the isolation of four acetylated pseudoguaianolides along with several known constituents. The structures of the compounds were derived from detailed studies of their spectral (1D and 2D NMR and FABMS) data and by comparison of the values with those of parthenin, a major known constituent of the plant. The cytotoxic activity of parthenin and the constituents was evaluated using Jurkat (human: T lymphocyte; acute T cell leukemia), HL-60 (human leukemia) and Hela (human cervical carcinoma) cells.

Keywords: *Parthenium hysterophorus*; Compositae; Acetylated pseudoguaianolide; Parthenin; Cytotoxic activity

1. Introduction

Parthenium hysterophorus Linn (Compositae), an obnoxious weed, grows wild in different regions of India. The plant causes contact dermatitis and allergic rhinitis in animals (Towers et al., 1977). It also possesses allelopathic activity (Kanchan, 1975; Patel and Hedge, 1988; Das and Das, 1995). Earlier chemical investigations on the plants reported the isolation of parthenin (**1**) (Herz et al., 1962), a pseudoguaianolide, as a major constituent along with some related sesquiterpenes and flavones (Romo de Vivar et al., 1966; Picman et al., 1980; Sethi et al., 1987; Dela Fuente et al., 1997; Venkataiah et al., 2003; Ramesh et al., 2003). The cytotoxic properties of various extracts and the constituents of the plant have been studied. The MeOH extract of the flowers showed significant antitumour activity (Mukherjee and Chatterjee, 1993) and parthenin exhibited cytotoxic properties (Kupchan et al., 1971).

In continuation of our work (Das et al., 1999; Venkataiah et al., 2003; Ramesh et al., 2003) on *P. hysterophorus* we report here the identification of four new acetylated pseudoguainanolides (**2**–**5**) isolated from a fresh collection of the flowers of the plant.

2. Results and discussion

The flowers of *P. hysterophorus* were extracted with $CHCl_3$–MeOH (1:1) and the extract was subjected to column chromatography over silica gel using hexane/EtOAc mixtures to yield parthenin (**1**) as the major constituent along with four new compounds **2**–**5** and the known constituents, coronopilin, tetraneurin A and hysterone D (Ramesh et al., 2003).

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Compound **2** was obtained as optically active colourless crystals. Its molecular formula $C_{17}H_{24}O_5$ was determined from elemental analysis, ^{13}C NMR spectrum and FABMS [*m*/*z* 326 ($M+NH_4^+$)]. The IR spectrum indicated the presence of hydroxyl and carbonyl groups. The structure of the compound was established from its ^1H and ^{13}C NMR spectral data (Tables 1 and 2) which were compared to those of parthenin (**1**). The assignments of protons and carbons were made with the help of 2D NMR (DQF-COSY, NOESY, HSQC and HMBC) and DEPT experiments (Fig. 1). The spectra revealed that the compound **2** was structurally related to **1**. Ring A of **2** contained the C-2, C-3 double bond and an acetate at C-4 (δ 2.13, 3H, s).

The DQF-COSY showed correlations between H-2 (δ 6.05, 1H, *dd*, $J = 5.9$, 2.1 Hz) and H-3 (δ 5.89, 1H, *dd*,$J = 5.9$, 1.4 Hz), H-3 and H-4 (δ 6.12, 1H, *dd*, $J = 2.1$, 1.4 Hz) and H-2 and H-4. In ring C, the exocyclic double bond was saturated to a methyl group (δ 2.36, 1H, m, H-11 and 1.23, 3H, *d*, $J = 7.0$ Hz, Me-13). The DQF-COSY clearly indicated the system H-7–H-11–Me-13. The ^{13}C NMR spectrum and the DEPT experiments also supported the above modifications of A and C rings. The B ring of both the compound **2** and parthenin was similar. The HMBC experiment showed that H-2 was related to C-4 (δ 83.7) and C-5 (δ 58.0), H-3 to C-1 (δ 87.5) and C-5, H-4 to C-2 (δ 138.4), C-6 (δ 83.8) and carbonyl group of the acetate (δ 171.0), H-6 (δ 5.22, 1H, $J = 9.0$ Hz) to C-4 and C-11 (δ 41.7) and H-7 (δ 2.54, m) to Me-13 (δ 15.4). In the NOESY Experiment, H-6 showed correlations with H-4 and H-7 while H-7 correlated with Me-13 but not with H-11. These correlations suggested that AcO-4 was β and Me-13 was α. The structure and stereochemistry of **2** was thus clearly decided. The X-ray crystallographic analysis of the compound (Fig. 2) clearly established its structure.

Table 2
^{13}C NMR data of **1**–**5**[a]

Carbon	1	2	3	4	5
1	84.8, (*s*)	87.5, (*s*)	88.8, (*s*)	154.1, (*s*)	156.2, (*s*)
2	163.4, (*d*)	138.4, (*d*)	139.11, (*d*)	125.6, (*d*)	125.7, (*d*)
3	131.5, (*d*)	125.1, (*d*)	135.4, (*d*)	73.6, (*d*)	73.8, (*d*)
4	211.2, (*s*)	83.7, (*d*)	85.1, (*d*)	207.2, (*s*)	207.8, (*s*)
5	59.2, (*s*)	58.0, (*s*)	58.0, (*s*)	58.0, (*s*)	56.6, (*s*)
6	78.8, (*d*)	83.8, (*d*)	85.9, (*d*)	80.0, (*d*)	80.5, (*d*)
7	44.7, (*d*)	46.5, (d)	44.5, (*d*)	43.3, (*d*)	43.0, (*d*)
8	28.4, (*t*)	24.6, (*t*)	21.3, (*t*)	23.5, (*t*)	25.3, (*t*)
9	30.2, (*t*)	27.6, (*t*)	31.2, (*t*)	29.8, (*t*)	28.8, (*t*)
10	40.0, (*d*)	42.9, (*d*)	40.9, (*d*)	38.9, (*d*)	38.5, (*d*)
11	140.5, (*s*)	41.7, (*d*)	38.6, (*d*)	138.1, (*s*)	139.4, (*s*)
12	170.8, (*s*)	179.9, (*s*)	179.9, (*s*)	169.4, (*s*)	169.7, (*s*)
13	121.6, (*t*)	15.4, (*q*)	10.7, (*q*)	120.1, (*s*)	120.7, (*s*)
14	17.7, (*q*)	13.1, (*q*)	14.2, (*q*)	14.6, (*q*)	17.5, (*q*)
15	18.2, (*q*)	16.7, (*q*)	17.9, (*q*)	20.7, (*q*)	21.4, (*q*)
OAc	–	21.3, (*q*)	20.0, (*q*)	20.6, (*q*)	20.6, (*q*)
–	–	171.0, (*s*)	171.0, (*s*)	170.1, (*s*)	168.9, (*s*)

[a] The spectra were run in $CDCl_3$ and the spectral data were assigned on the basis of DEPT, HSQC and HMBC experiments.

Compound **3** was isolated as a colourless viscous mass. It analyzed for $C_{17}H_{24}O_5$ from elemental analysis, ^{13}C NMR spectrum and FABMS (*m*/*z* 326 [$M+NH_4^+$]). The molecular formula of the compound was similar to that of **2**. The IR spectrum indicated the presence of hydroxyl and carbonyl groups in the molecule. The ^1H and ^{13}C NMR spectral data of **3** (Tables 1 and 2) suggested that its structure was similar to that of **2**. The ring A contained a double bond at C-2, C-3 and an acetoxy group at C-4

Table 1
^1H NMR data of **1**–**5**[a,b]

Proton	1	2	3	4	5
2	7.48, *d*, (6.2)	6.05, *dd*, (5.9, 2.1)	5.97, *dd*, (6.1, 2.1)	6.02, *d*, (1.5)	6.08, *d*, (1.5)
3	6.15, *d*, (6.2)	5.89, *dd*, (5.9, 1.4)	5.92, *dd*, (6.1, 1.5)	5.93, *d*, (1.5)	5.44, *d*, (1.5)
4	–	6.12, *dd*, (2.1, 1.4)	6.15, dd, (2.1, 1.5)	–	–
6	4.98, *d*, (8.2)	5.22, *d*, (9.0)	4.82, *d*, (9.0)	4.46, *d*, (9.0)	4.62, *d*, (9.0)
7	3.46, *m*	2.54, *m*	3.05, *m*	3.42, *m*	3.38, *m*
8	2.37–2.18, *m*,	2.09–2.02, *m*, 1.86, *m*,	1.76, *m*, 1.51, *m*,	2.12, *m*, 2.02, *m*	2.05, *m*, 1.96, *m*
9	1.84, *m*, 1.63, *m*	2.09–2.02, *m*, 1.62, *m*	2.08, *m*, 1.67, *m*	1.84, *m*, 1.75, *m*	1.81, *m*, 1.63, *m*
10	2.10, *m*	2.21, *m*	2.21, *m*	2.91, *m*	2.94, *m*
11	–	2.36, *m*	2.84, *m*	–	–
13	6.24, *d*, (2), 5.56, *d*, (2)	1.23, *d*, (7)	1.16, *d*, (7)	6.28, *d*, (2) 5.55, *d*, (2)	6.24, *d*, (2), 5.58, *d*, (2)
14	1.24, *s*	1.08, *s*	1.07, *s*	1.21, *s*	1.24, *s*
15	1.12, *d*, (7)	1.09, *d*, (7)	1.10, *d*, (7)	1.19, *d*, (7)	1.19, *d*, (7)
OAc	–	2.13, (*s*)	2.13, *s*	2.12, *s*	2.12, *s*

[a] The spectra were run in $CDCl_3$ and the spectral data were assigned on the basis of DQF-COSY, NOESY and HMBC experiments.
[b] *J* values (in Hz) are in parenthesis.

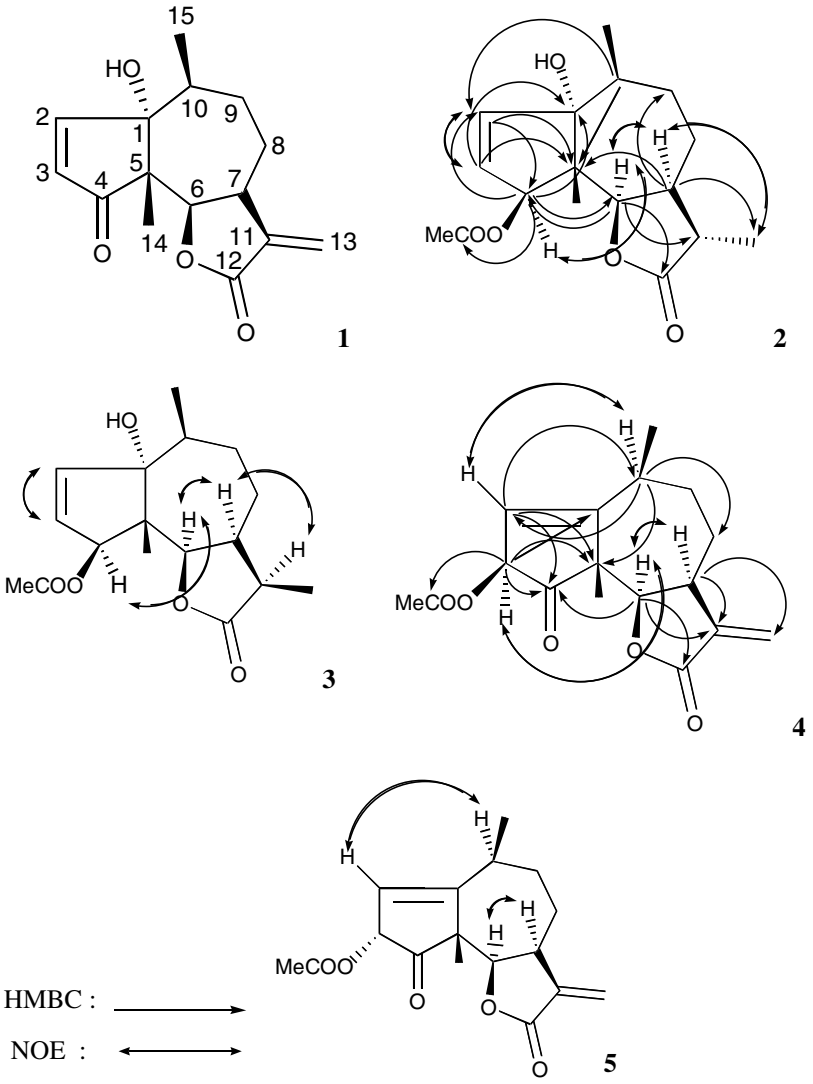

Fig. 1. Selected HMBC and NOESY correlations of **1**–**5** (HMBC of **2** and **3** and of **4** and **5** were similar).

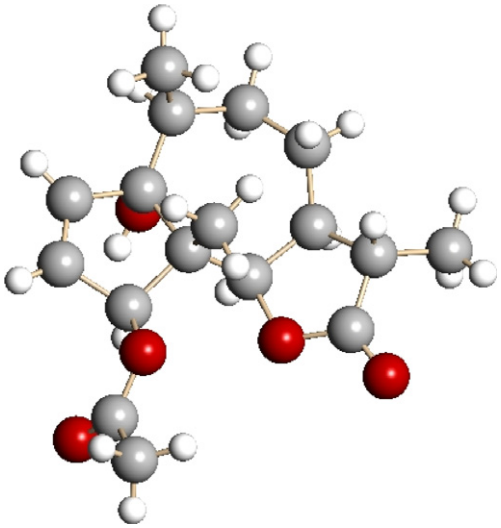

Fig. 2. Crystal structure of compound **2**.

while ring C contained a methyl group at C-11. The DQF-COSY and HMBC experiments also clearly suggested this (Fig. 1). In the HMBC experiment, H-4 (δ 6.12, *dd*, $J = 2.1$, 1.4 Hz) showed correlation with the carbonyl group of acetate (δ 171.0) indicating the acetylation of the –OH group at C-4. A NOESY experiment showed that H-6 (δ 4.82, 1H, *d*, $J = 9.0$ Hz) correlated with H-4 (δ 6.15, 1H, *dd*, $J = 2.1$, 1.5 Hz) and H-7 (δ 3.05, 1H, m) while H-7 to H-11 (δ 2.84, 1H, m). Thus the acetoxy group at C-4 and the methyl at C-11 had both a β-configuration. Consequently, the structure of **3** was clearly established as the C-11 epimer of **2**.

Compound **4** was obtained as a colourless viscous mass. Its molecular formula was deduced to be $C_{17}H_{20}O_5$ from elemental analysis, ^{13}C NMR spectrum and FABMS (*m*/*z* 322 $[M+NH_4^+]$). The IR spectrum showed that the molecule contained carbonyl groups. The ^1H and ^{13}C NMR spectral data of **4** (Tables 1 and 2) suggested that its structure was related to parthenin but the A ring of these two compounds was different. In **4**, the ring A contained

a double bond at C-1, C-2 as well as an acetoxy and a carbonyl groups at C-3 and C-4, respectively (Tables 1 and 2). The DQF-COSY experiment showed the correlation between H-2 (δ 6.02, 1H, *d*, $J = 1.5$ Hz) and H-3 (δ 5.93, 1H, *d*, $J = 1.5$ Hz). Ring C of **4** having an exocyclic double bond at C-11, C-13 (δ 6.28 and 5.55, 1H each, *d*, $J = 2.0$ Hz) was structurally similar to that of parthenin (**1**). The HMBC spectrum showed that H-2 was related to C-4 (δ 207.2), C-5 (δ 58.0) and C-10 (δ 38.9), H-3 to C-1 (δ 154.7), C-5 and carbonyl group (δ 170.1) of –OAc, H-6 (4.46, 1H, *d*, $J = 9.0$ Hz) to C-4, C-11 (δ 138.1) and C-12 (δ 169.4) and H-7 to C-13 (δ 120.1) (Fig. 1). In the NOESY experiment, H-6 showed correlations with H-3 (δ 5.93, 1H, d, $J = 1.5$ Hz) and H-7 (δ 3.42, m) suggesting the β-configuration of the acetoxy group at C-3. The structure and stereochemistry of **4** was thus clearly established.

Compound **5** was also isolated as a colourless viscous mass. Its molecular formula was assigned to be $C_{17}H_{20}O_5$ from elemental analysis, ^{13}C NMR spectrum and FABMS (*m*/*z* 322 $[M+NH_4^+]$). The molecular formula of the compound was similar to that of **4**. The IR spectrum indicated the presence of carbonyl groups in the molecule. The ^1H and ^{13}C NMR spectral data of **5** (Tables 1 and 2) revealed that its structure was similar to that of **4**. The ring A of **5** contained a double bond at C-1, C-2, an acetoxy group at C-3 and a carbonyl group at C-4 while ring C contained an exocyclic double bond at C-11, C-13. This was also suggested by the DQF-COSY and HMBC experiments (Fig. 1). However, in NOESY experiment H-6 (δ 4.62, 1H, *d*, $J = 9.0$ Hz) did not show any correlation with H-3 (δ 5.44, 1H, *d*, $J = 1.5$ Hz) but it showed correlation with H-7 (δ 3.38, 1H, m) indicating the α-configuration of the acetoxy group at C-3. The structure of **5** was thus determined as the C-3 epimer of **4**.

The known constituents, coronopilin, tetraneurin A and hysterone D were characterized by comparison of their physical (m.p. and $[\alpha]_D$) and spectral (^1H and ^{13}C NMR and MS) properties with those of the authentic samples (Ramesh et al., 2003). All the isolated compounds were originally present in plant extract as determined by TLC.

To determine growth inhibitory effect of isolated constituents of *P. hysterophorus* on human cancer cells, we performed cell viability assays (*in vitro* cytotoxic activity analyzed by MTT assays) using three human cancer cell lines. It is evident from Table 3, parthenin and isolated new compounds decreased cell growth in cultured human cancer cells (HL-60, Hela and Jurkat) in a concentration dependent manner. The present results demonstrate for the first time that parthenin and related structures inhibits the proliferation of Jurkat (human T-lymphocyte leukemia) cells by triggering apoptosis. Therefore, we further examined the cytotoxic effects of these structures in human leukemia HL-60 and human cervical carcinoma Hela cells. All the isolated compounds have shown promising cytotoxic activity against Jurkat cells followed by moderate activity on HL-60. However, **2**–**5** exhibited insignificant activity on Hela cells.

Table 3
Cytotoxic activity of parthenin and its analogues on cancer cell growth *in vitro*

Test compound	IC_{50} (μM)		
	Jurkat (acute T cell leukemia)	HL-60 (human leukemia)	Hela (human cervical carcinoma)
Parthenin (**1**)[a]	0.061 ± 0.001	156.79 ± 20.42	594.16 ± 42.35
2	40.62 ± 3.51	320.13 ± 28.21	748.79 ± 38.47
3	14.64 ± 4.51	396.23 ± 37.27	1956.36 ± 57.68
4	16.28 ± 1.53	237.17 ± 18.56	1045.33 ± 23.58
5	28.16 ± 2.68	244.44 ± 11.63	715.95 ± 32.47

[a] Positive control.

3. Experimental

3.1. General

Melting point was measured in a Buchi-510 instrument and is uncorrected. Spectra were recorded with the following instruments: IR: Perkin–Elmer spectrophotometer; ^1H and ^{13}C NMR; Bruker Aviance 600 MHz and MS: Finnigan MAT 1020 instrument. Optical rotations were measured on a JASCO DIP-360 digital polarimeter. Chromatography was performed on silica gel (BDH 100–200 mesh) and TLC with silica gel GF 254.

3.2. Plant material

The flowers of *P. hysterophorus* were collected from campus in the month of September, 2005 and were botanically identified. A Voucher specimen (No. IICT-5406) was preserved in the herbarium of the institute.

3.3. Extraction and isolation

The air-dried and powdered plant material (2 kg) was extracted thrice with CH_2Cl_2–MeOH (1:1) at room temperature. Each extraction was performed for 72 h using 4 L of the solvent. The combined extract was concentrated under reduced pressure to afford a brown gummy residue (33 g). The residue was subjected to column chromatography using solvents of increasing polarity from *n*-hexane through EtOAc. Four fractions, A–D were eluted with hexane-EtOAc, 80:20, 70:30, 60:40 and 40:60, respectively. The fractions A and B afforded coronopillin (29 mg) and parthenin (13.8 g), respectively. Fractions C was found to contain two compounds and fraction D four compounds. These fractions were further purified by repeated column chromatography using a mixture of hexane and EtOAc. Fraction C furnished tetraneurin A (36 mg) and hysterone D (16 mg) while fraction D yielded compounds **2** (32 mg), **3** (25 mg), **4** (28 mg) and **5** (18 mg). (eluted with hexane-EtOAc, 55:45, 50:50, 45:55, and 40:60, respectively). Com-

pound **2** crystallized from CH_2Cl_2–hexane (90:10). The other new compounds **3**–**5** were obtained as colourless viscous materials.

*3.3.1. Compound **2***

Colourless crystals, m.p. 177–178 °C, $[\alpha]_D^{25}$ −31.4 (c 0.5, $CHCl_3$); IR: ν_{max} (KBr) 3493, 1744, 1456, 1377, 1245, 1041, 767 cm^{-1}; ^1H and ^{13}C NMR: Tables 1 and 2 ; FABMS: *m/z* 326 $[M+NH_4^+]$; Anal. Calc. for $C_{17}H_{24}O_5$: C, 66.23; H, 7.79%. Found: C, 66.36; H, 7.83%.

*3.3.2. Compound **3***

Colourless viscous mass, $[\alpha]_D^{25}$ −21.1 (c 0.4, $CHCl_3$); IR: ν_{max} (KBr) 3496, 1744, 1448, 1245, 1027, 765 cm^{-1}; ^1H NMR and ^{13}C NMR: Tables 1 and 2; FABMS: *m/z* 326 $[M+NH_4^+]$; Anal. Calc. for $C_{17}H_{24}O_5$: C, 66.23; H, 7.79%. Found: C, 66.17; H, 7.83%.

*3.3.3. Compound **4***

Colourless viscous mass, $[\alpha]_D^{25}$: +1.8 (c 0.5, $CHCl_3$); IR: ν_{max} (KBr) 1769, 1618, 1372, 1014, 969 cm^{-1}; ^1H and ^{13}C NMR: Tables 1 and 2; FABMS: *m/z* 322 $[M+NH_4^+]$; Anal. Calc. for $C_{17}H_{20}O_5$: C, 67.11; H, 6.58%. Found: C, 67.04; H, 6.63%.

*3.3.4. Compound **5***

Colourless viscous mass, $[\alpha]_D^{25}$: −93.1 (c 0.4, $CHCl_3$); IR: ν_{max} (KBr) 1767, 1619, 1372, 1222, 1013, 756 cm^{-1}; ^1H and ^{13}C NMR: Tables 1 and 2; FABMS: *m/z* 322 $[M+NH_4^+]$; Anal. Calc. for $C_{17}H_{20}O_5$: C, 67.11; H, 6.58%. Found: C, 67.24; H, 6.49%.

*3.4. X-ray crystallographic analysis of **2***

Compound **2** crystallized from CH_2Cl_2–hexane (90:10). The crystal system was orthorhombic and space group was $P2_12_12_1$. The unit cell dimensions are as follows: $a = 6.9450(4)$ Å, $b = 9.1985(6)$ Å, $c = 25.9912(16)$ Å, $\alpha = 90°$, $\beta = 90°$ and $\gamma = 90°$. The crystal size was $0.19 \times 0.15 \times 0.07$ mm. The reflections collected were 12019 and the independent reflections were 1713. The refinement method was full-matrix least squares on F^2.

3.5. Cytotoxicity studies

Jurkat, HL-60 and Hela cells were obtained from NCCS, Pune, India and were cultured at 37 °C with 5% CO_2, using RPMI-1640 media containing 10% fetal bovine serum in the presence or absence of test compounds. MTT assay was used to study the cytotoxic properties of the evaluated samples. The assay was performed *in vitro* using a modified method described by Plumb et al. (1989). The cells (2×10^4) were seeded in each well plate. After incubation for 24 h the test compound with different concentrations (in μM) were added to respective wells and after further 24 h, 10 μl of MTT (5 mg/ml stock solution, Sigma) was added to each well. The medium was discarded after 4 h and the formazan blue, which formed in the cells, were dissolved with 100 μl of DMSO. The rate of color production was measured at 570 nm in a spectrophotometer (Spectra MAX Plus supported by SOFT max PRO-3.0). All experiments were conducted under the standard laboratory illumination. The percentage of inhibition of cell viability was computed with reference to the MTT reduction in control (without test compound). The experimental measurements were made in five replicates each and the average value was taken as percentage inhibition. The data were subjected to linear regression analysis and the regression lines were plotted for the best straight line fit. The IC_{50} values were calculated based on these straight lines following the referred method.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2007.05.002.

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