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Two new cytotoxic labdane diterpenes from the rhizomes of *Hedychium coronarium*

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ABSTRACT

The phytochemical investigation of the hexane extract of the *Hedychium coronarium* led to the isolation and identification of two new labdane diterpenes (**1** and **2**) along with 10 known metabolites (**3**–**12**). The structures of the new compounds were established on the basis of spectroscopic data analysis (1D and 2D NMR and MS). Cytotoxic activities of the isolates were studied against the A-549 (lung cancer), SK-N-SH (human neuroblastoma), MCF-7 (breast cancer) and HeLa (cervical cancer) cell lines.

The genus *Hedychium* (Zingiberaceae family) is an economically important genus that comprises about 80 species, a number of which are used as traditional herbal medicines throughout the world.[1] Recently, much attention has been paid to *Hedychium* species due to their diverse biological activities such as anti-inflammatory,[2] anti-tumour,[3] anti-allergic,[4] analgesic,[5] anti-helmintic[6] and significant cytotoxic effects.[7] Plants of the *Hedychium* species are rich source of sesquiterpenes, diterpenes with a wide range of biological and pharmacological activities.[8] Their structural features comprise a fundamental skeleton of decalone moiety with a variety of substituents, could be classified to different types such as furanolabdanes, prefuranoid labdanes which include hemiacetals/spiroethers and lactone rings.[8] In general, the labdane skeleton shown by hedychenone is a typical marker of the genus *Hedychium*.

Hedychium coronarium, popularly known 'butterfly ginger' is widely available in tropical and subtropical regions, such as Japan, India, Brazil, South China, Southeast Asian countries and so on. Its rhizomes and fruits have been used in folklore medicine for the treatment of arthritis, diabetes, headache, and hypertension.[9] A survey of the literature revealed that several labdane-type diterpenes and farnesane-type sesquiterpenes were isolated from this species.[10] As part of our continuing interest in search of biologically active labdane diterpenes from *Hedychium* species that are used in folk medicine,[11] we herein describe the isolation and structure elucidation of two new labdane diterpenes (**1** and **2**) and ten known compounds (**3**–**12**) from the rhizomes of *H. coronarium* (Fig. 1). Among the known compounds, compounds **6**–**8** and **12** were isolated for the first time from this plant. The cytotoxicity of several of these compounds was also evaluated against the A-549 (lung cancer), SK-N-SH (human neuroblastoma), MCF-7 (breast cancer) and HeLa (cervical cancer) cell lines.

The rhizomes of *H. coronarium* (10 kg) were shade dried, powdered and extracted with hexane in a soxhlet apparatus for 72 h. The resulting hexane extract was evaporated to dryness under reduced pressure, affording syrupy residue (5 g). Then this hexane extract was subjected to column chromatography on a silica gel column (60–120 mesh, 150 × 15 cm) and eluted with a step wise gradient of hexane/EtOAc (99:1, 98:2, 92:8, 90:10, 88:12 by volume) to afford a total of 90 fractions of 50 ml each. Column fractions were analyzed by TLC (Silica Gel 60 F254, hexane/EtOAc, 70:30), and fractions with similar TLC patterns were combined to give five major fractions (F_1, F_2, F_3, F_4, and F_5). Fraction F_1 was subjected to repeated silica gel (100–200 mesh) column chromatography (CC) by eluting with EtOAc/hexane (2:98) to yield compound **11** (1.89 g), with EtOAc/hexane (3:97) to yield compound **10** (0.9 g) and with EtOAc/hexane (5:95) to yield compound **5** (0.1 g), respectively. A portion of fraction F_2 was subjected to silica gel column chromatography with EtOAc/hexane (5:95) to yield sub fractions F_{2a} and F_{2b}. Fraction F_{2a} was subjected to repeated chromatography over silica gel to get the compounds **4** (0.05 g) and **3** (0.120 g). Further, subfraction F_{2b} was eluted with EtOAc/ether/hexane (18:5:77) to yield compound **6** (0.2 g) and **7** (0.85 g).

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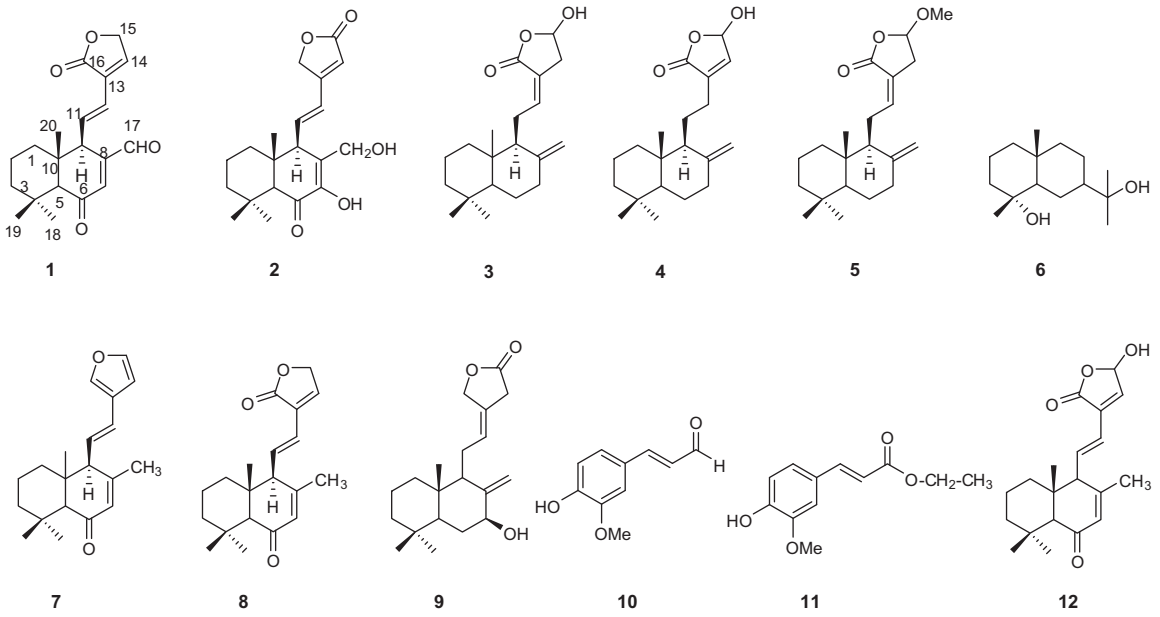

Figure 1. Compounds isolated from *Hedychium coronarium*.

Similarly, fraction F_3 was subjected to repeated column chromatography eluting with EtOAc/hexane (5:95) followed by preparative TLC yielded 0.042 g of compound **1**, with EtOAc/hexane (5.5:94.5) to yield 0.085 g of compound **2.** Fraction F_4 was subjected to repeated column chromatography eluting with EtOAc/ether/hexane (29:10:61) to yield 0.135 g of compound **8**, with EtOAc/ether/hexane (30:10:60) to yield 0.095 g of compound **9** and with EtOAc/ether/hexane (25:10:65) to get the compound **12** (0.08 g), respectively.

Compound **1** was obtained as pale orange oil with optical rotation $[\alpha]_D^{25} = +105$ (*c* 0.5, MeOH). The molecular formula was determined as $C_{20}H_{24}O_4$ by HRESIMS, which provided a molecular ion peak at *m/z* 329.1053 $(M+H)^+$ [calcd for $C_{20}H_{25}O_4$, 329.1078], in conjunction with its ^{13}C NMR spectrum. The IR spectrum indicated the presence of an α,β-unsaturated γ-lactone ring (1730 cm^{-1}), α,β-unsaturated C=O (1672 cm^{-1}) and aldehyde (1685 cm^{-1}) functional groups. The ^1H NMR spectrum in $CDCl_3$ (Table 1) exhibited signals for three methyl groups [δ 1.25, 1.19, 1.05] and two methine groups [δ 2.21 (s, H-5), 3.25 (*d*, *J* = 9.8 Hz, H-9)], which were characteristic of labdane diterpene skeleton. The olefinic protons at δ 6.78 (dd, *J* = 15.8, 9.8 Hz), 6.29 (d, *J* = 15.8 Hz) and at δ 6.52 (s, 1H) were attributed to H-11, H-12, and H-7, respectively. In addition, ^1H NMR spectrum (Table 1) indicated presence of an aldehyde group [δ 9.73 (s, H-17)] and an oxymethylene group [4.88 (2H, d, *J* = 2.0 Hz)]. ^{13}C NMR spectrum of **1** (Table 1), together with the information from a DEPT & HSQC spectrum, showed the presence of 20 carbon signals assigned to three methyls (tertiary), four methylenes (three from decalone moiety and one from lactone

Table 1
^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) spectral data of **1** and **2**

Position	Compound **1**		Compound **2**	
	δ_c	δ_H (multiplicity)	δ_c	δ_H (multiplicity)
1	39.62	1.54 (2H, m)	40.09	1.59 (2H, m)
2	17.85	1.42 (2H, m)	18.02	1.46 (2H, m)
3	42.92	1.26 (2H, m)	43.24	1.28 (2H, m)
4	22.82	—	22.68	—
5	64.09	2.21 (1H, s)	64.12	2.11 (1H,s)
6	200.27	—	199.49	—
7	139.56	6.52 (1H, s)	157.19	—
8	149.82	—	144.36	—
9	54.81	3.25 (1H, d, *J* = 9.8 Hz)	59.13	3.01 (1H, d, *J* = 9.8 Hz)
10	31.92	—	31.91	—
11	132.71	6.78 (1H, dd, *J* = 15.8, 9.8 Hz)	133.10	6.78 (1H, dd, *J* = 15.8, 9.8 Hz)
12	123.08	6.29 (1H, d, *J* = 15.8 Hz)	123.71	6.31 (1H, d, *J* = 15.8 Hz)
13	128.76	—	128.16	—
14	144.03	7.25 (1H, t, *J* = 2.0 Hz)	125.16	6.10 (1H, s)
15	69.69	4.88 (2H, d, *J* = 2.0 Hz)	172.05	—
16	172.01	—	69.71	4.84 (2H, s)
17	194.11	9.73 (1H, s)	63.69	4.02 (1H, d, *J* = 17.0 Hz) 4.16 (1H, d, *J* = 17.0 Hz)
18	33.16	1.25 (3H, s)	33.47	1.38 (3H, s)
19	21.73	1.19 (3H, s)	21.63	1.21 (3H, s)
20	15.73	1.05 (3H, s)	15.83	0.98 (3H, s)

Assignments were based on 2D NMR including DQF-COSY, HSQC, HMBC and NOESY. Well-resolved couplings are expressed with coupling patterns and coupling constants in Hertz in parentheses. For overlapped signals, only chemical shift values are given.

ring), four vinylic, two methines, six non protonated carbons (two carbonyl, two olefinic and two quaternary carbons) and an aldehyde.

Careful analysis and comparison of the NMR data of **1** (see Table 1) with that of **8** revealed that both compounds were based on the same carbon skeleton.[12] Typical difference in 1D NMR spectra of **1** was that the methyl group of **8** was replaced by the aldehyde group in the decalone ring. The complete assignments of all proton resonances of **1** are achieved, using DQF-COSY, HSQC and HMBC experiments (Fig. 2). From the ^{1}H–^{1}H COSY spectrum, key correlations were seen between H-1 [δ 1.54 (m)] to H-2 [δ 1.42 (m)] and H-2 [δ 1.42 (m)] to H-3 [δ 1.26 (m)], which can be assigned to mutually coupled methylene protons of decalone ring. This evidence coupled with the HMBC correlations of H-1/C-2, C-10; H-2/C-1, C-3; H-3/C-2, C-4; Me-19/C-4; Me-18/C-4, C-3, C-5, Me-20/C-1, C-10, C-9 confirmed the decalone ring. In the ^{13}C NMR spectrum, signal at δ 200.27 was assigned to α,β-unsaturated carbonyl group at C-6, corresponding with the HMBC cross-peaks between H-5 (δ 2.21) /C-6 (δ 200.27). The position of the aldehyde group at C-17 was supported by HMBC correlations from H-17/C-7, C-8 and C-9. A cross-peak between the signals at δ 7.25 (H-14) and δ 4.88 (H-15) in ^{1}H–^{1}H COSY experiments (Fig. 2) led to propose the position of the carbonyl group at C-16 in the lactone ring, which was confirmed by the observation of HMBC correlations of H-14/C-15 (δ 69.69), C-13 (δ 128.76) and H-15/C-16 (δ 172.01). Furthermore, The HMBC correlations of δ 3.25 (H-9) with δ 132.71 (C-11), δ 123.08 (C-12) and δ 149.82 (C-8) established the connection between the decalone ring and γ-lactone moiety (Fig. 2).

The relative stereochemistry of **1** was achieved by analysis of its NOESY spectrum (Fig. 3). The configuration of the decalone portion of **1** was assumed to be the same as that of known diterpenes bearing the same skeleton such as hedychenone.[13] NOESY spectrum showed the correlations between Me-19/Me-20; H-5/H-9 and H-5/Me-18. These data were in agreement with the β-orientation of Me-19, Me-20 and α-orientation of H-5, Me-18 and H-9. The large coupling constant (J = 15.8 Hz) of H-11 and H-12 indicated the *trans* relationship of these protons. On the basis of these data, the structure of compound **1** was established as 6-oxo-7,11,13-labdatrien-17-al-16,15-olide.

Compound **2** was obtained as white amorphous resin with optical rotation $[\alpha]_D^{25} = +98$ (c 0.5, MeOH) and its molecular composition determined as $C_{20}H_{26}O_5$ based on the sodiated molecular ion peak at m/z 369.3823 $[M+Na]^+$ (calcd for $C_{20}H_{26}O_5Na$, 369.3825) in the HRESIMS. The structural characterization of **2** was carried out in an analogous manner.[14] A side-by-side comparison between the NMR spectra of **1** and **2** rapidly pinpointed their structural similarities. The ^{1}H NMR and ^{13}C NMR spectroscopic data of the compound **2** (Table 1) indicated labdane skeleton, which is similar to that of compound **1**; both have α,β-unsaturated ketone (200.27 in **1**, 199.49 in **2**), lactone ring (172.01 in **1**, 172.05 in **2**) and trans double bond (δ 132.71, 123.08 in **1**; δ 133.10, 123.71 in **2**) directly attached to the decalone nucleus. The major difference in ^{1}H and ^{13}C NMR (Table 1) spectra of **2** indicated the lack of a proton signal at δ 6.52 (H-7), which is replaced by a hydroxyl group, as revealed by the molecular formula. Another difference was that the presence of oxymethylene protons [δ 4.02 (1H, d, J = 17 Hz), 4.16 (1H, d, J = 17 Hz)] in **2** instead of aldehyde group in **1**. Corresponding evidence in ^{13}C NMR and DEPT was found from the resonances for the oxymethylene carbon at δ 63.69 and the key HMBC correlations of H-17/C-8 (δ 144.36), C-7 (δ 157.19) and C-9 (δ 59.13). Further, a sharp singlet at δ 4.84 (H-16) integrating for two protons and a one proton singlet at δ 6.10 (s, H-14) were ascribed to lactone ring protons. The absence of ^{1}H–^{1}H COSY correlations between H-14 and H-15 coupled with the HMBC correlations of H-14 (δ 6.10)/C-15 (δ 172.05); H-16 (δ 4.84)/C-15 (δ 172.05), C-13 (δ 128.16) confirmed the position of the carbonyl group at C-15 in the lactone ring.

The complete assignment of the labdane skeleton frame work was achieved through the interpretation of COSY, HSQC, HMBC and NOESY correlations. In the HMBC spectrum (Fig. 4), following major correlations were observed regarding its carbon skeleton: H-5 (δ 2.11)/C-4 (δ 22.68), C-6 (δ 199.49), C-10 (δ 31.91); H-9 (δ 3.01)/C-8 (δ 144.36), C-10 (δ 31.91), C-11 (δ 133.10); H-11 (δ 6.78)/C-9 (δ 59.13), C-12 (δ 123.71); H-12 (δ 6.31)/C-11

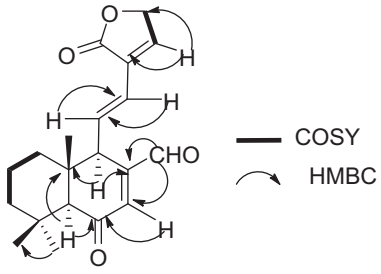

Figure 2. Key COSY and HMBC correlations of compound **1**.

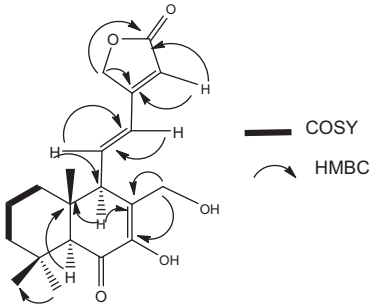

Figure 4. Key COSY and HMBC correlations of compound **2**.

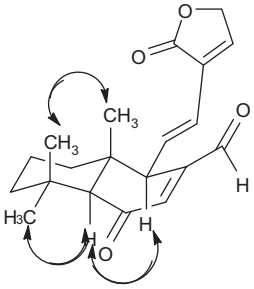

Figure 3. NOESY correlations of compound **1**.

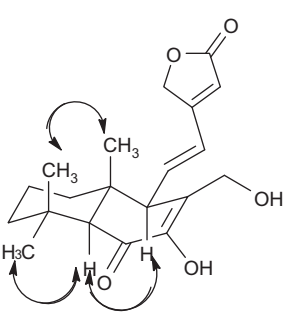

Figure 5. NOESY correlations of compound **2**.

Table 2
GI_{50} and LC_{50} values (in μM) for the compounds **1–12**

Compound	A-549		SK-N-SH		MCF-7		HeLa	
	GI_{50} (μM)	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}
1	18.5 ± 0.95	45.9 ± 1.9	38.7 ± 4.2	48.5 ± 3.8	69.9 ± 8.5	>100	76.9 ± 6.2	>100
2	26 ± 1.7	48.7 ± 4.4	12.1 ± 1.6	19.3 ± 2.5	12.0 ± 1.4	15.4 ± 0.9	79.7 ± 5.8	>100
3	8 ± 0.95	14 ± 1.07	20.1 ± 2.6	32.0 ± 3.6	17.4 ± 2.1	21.9 ± 1.8	47.0 ± 5.6	60.6 ± 7.3
4	4.8 ± 0.19	9.5 ± 0.88	25.8 ± 3.2	33.7 ± 2.8	32.6 ± 3.6	40.7 ± 3.4	36.3 ± 4.3	45.9 ± 3.6
5	30 ± 3.9	71.3 ± 6.28	13.6 ± 1.8	19.1 ± 2.1	25.3 ± 2.8	31.6 ± 2.5	>100	>100
6	14.5 ± 0.2	27.4 ± 4.52	73.6 ± .8	90.9 ± 10.3	62.3 ± 7.6	87.0 ± 6.9	75.6 ± 8.9	>100
7	7.4 ± 0.65	14 ± 1.9	11.6 ± 1.5	17.2 ± 2.2	14.9 ± 2.3	19.8 ± 1.3	63.2 ± 5.4	81.5 ± 9.7
8	21.7 ± 0.44	39.6 ± 0.9	8.2 ± 1.1	20.8 ± 2.4	15.7 ± 2.4	19.8 ± 15	83.6 ± 6.7	>100
9	15.1 ± 0.5	36.2 ± 4.48	54.8 ± 4.7	59.9 ± 6.7	44.7 ± 5.3	55.6 ± 4.5	62.1 ± 4.7	80.7 ± 6.7
10	1.7 ± 0.02	11.4 ± 0.3	10.6 ± 1.3	16.1 ± 1.4	39.5 ± 3.5	48.5 ± 3.4	61.8 ± 5.2	79.0 ± 9.5
11	1.2 ± 0.01	5.8 ± 0.5	22.3 ± 2.5	31.4 ± 2.4	86.3 ± 9.4	>100	52.8 ± 3.7	67.8 ± 5.4
12	10.6 ± 0.65	21.2 ± 3.3	17.5 ± 1.5	22.8 ± 1.8	28.5 ± 2.5	34.8 ± 4.2	>100	>100
Nocodozole	<0.1	<0.1	0.66	0.75	<0.1	<0.1	0.75	0.9

(δ 133.10); H-14 (δ 6.10)/C-15 (δ 172.05); H-16 (δ 4.84)/C-13 (δ 128.16), C-15 (δ 172.05); H-17 (δ 4.02, 4.16)/C-8 (δ 144.36); H-5 (δ 2.11), Me-18 (δ 1.38), H-3 (δ 1.28), Me-19 (δ 1.21)/C-4 (δ 22.68).

The relative stereochemistry at chiral centers was consistent with that of **1** based on the analysis of NOESY spectrum as well as biogenetic considerations (Fig. 5). NOE effects between Me-19/ Me-20; H-5/H-9, and H-5/ Me-18 suggested the β-orientation of H_3-19, H_3-20 and α-orientation of H-18, H-5 and H-9. On the basis of these spectral data, compound **2** characterized as 7,17-dihydroxy-6-oxo-7,11,13-labdatrien-16,15-olide.

By comparing physical and spectroscopic data with literature values, the eight known constituents (**3**–**12**) were identified as coronarin D (**3**),[7] coronarin C (**4**),[7] coronarin D methyl ether (**5**),[15] cryptomeridiol (**6**),[16] hedychenone (**7**),[13] 6-oxo-7,11,13-labdatriene-16,15-olide (**8**),[12] pacovatinin A (**9**),[17] 4-hydroxy3-methoxy cinnamaldehyde (**10**),[18] 4-hydroxy-3-methoxy ethyl cinnamate (**11**),[19] and **12**[20].

All the 12 compounds (**1**–**12**) were examined with respect to their growth inhibitory properties against the A-549, SK-N-SH, MCF-7 and HeLa cancer cell lines. The screening procedure was based on the standard Sulforhodamine B (SRB) assay.[21] Cell line assays were performed using three different concentrations of compounds (10^{-5}, 10^{-6}, 10^{-7} M) in triplicates. The LC_{50} and GI_{50} values were expressed as mean ± standard deviation (Table 2) and compared with the nocodozole.[22] As observed from Table 2, structural differences of the labdane diterpenes significantly affected the anticancer activity. Among the test compounds, 4-hydroxy-3-methoxy ethyl cinnamate (**11**), 4-hydroxy-3-methoxycinnamaldehyde (**10**), hedychenone (**7**), coronarin C (**4**) and coranarin D (**3**) displayed potent cytotoxic activity against A-549 cell line with the LC_{50} value ranging from 1.26 to 8.0 μM, respectively. Two new compounds **1** and **2** showed moderate cytotoxicity on the tested cell lines.

In summary, in continuation of our pharmacological–phytochemical integrated studies on the plants of the genus *Hedychium*, we have investigated *H. coronarium* rhizomes which resulted in the isolation of two new compounds (**1** and **2**) with moderate cytotoxic activity. Based on these preliminary reports obtained by us, further studies directed towards polar constituents of this plant to identify potential anticancer leads.

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14. *Compound* **1**: IR (KBr) ν_{max} 1730 (lactone), 1685 (aldehyde), 1672 (α,β-unsaturated ketone), 1628 (olefinic) and 1360 cm^{-1}. HRESIMS at *m/z* $[M+H]^+$ 329.1053. $[\alpha]_D^{25} = +105$ (*c* 0.5, MeOH). *Compound* **2**: IR (KBr) ν_{max} 1745 (lactone), 1662 (α,β-unsaturated ketone), 1624 (olefinic), 1370 cm^{-1}. HRESIMS at *m/z* 369.3823 $[M+Na]^+$. $[\alpha]_D^{25} = +98$ (*c* 0.5, MeOH).
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22. *Experimental procedure for cytotoxic activity*: compounds (**1**–**12**) were evaluated for their in vitro cytotoxicity in four different human cancer cell lines (A549, MCF-7, HeLa and SK-N-SH). A protocol of 48 h continuous drug exposure has been used and a sulforhodamine B (SRB) protein assay has been employed to estimate cell viability or growth. The cell lines were grown in Minimal Essential Medium containing 10% fetal bovine serum and 2 mM of L-glutamine and were seeded into 96-well microtiter plates in 90 μL volume (~60% confluent). The microtiter plates were incubated at 37 °C, 5% CO_2, 95% air for 12 h prior to addition of experimental drugs. Aliquots of 10 μL of the drug dilutions were added to cells resulting in the required final drug concentrations. For each compound three concentrations (0.1, 1 and 10 μM) were evaluated and each was done in triplicate wells. Plates were incubated further for 48 h and assay was terminated by the addition of 50 μL of cold trichloro acetic acid (TCA) (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The plates were washed five times with water and air-dried. Sulforhodamine B (SRB) solution (50 μL) at 0.4% (w/v) in 1% acetic acid was

added to each of the wells, and plates were incubated for 20 min at room temperature. The residual dye was removed by washing five times with 1% acetic acid. The plates were air-dried and the bound stain was subsequently eluted with 10 mM Trizma base. The absorbance was read on an ELISA plate reader at a wavelength of 540 nm with 690 nm reference wavelengths. Percent growth was calculated on a plate by plate basis for test wells relative to control wells as described earlier. The potency of the compounds was compared with that of positive control, nocodozole.