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Original article

Bakuchiol derivatives as novel and potent cytotoxic agents: A report

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ABSTRACT

A library of 28 compounds comprising of acyl, amino, halo, nitro, styryl and cyclized derivatives of bakuchiol have been evaluated against a panel of eight human cancer cell lines. Bioevaluation studies have resulted in the identification of potent cytotoxic molecules exhibiting concentration dependent growth inhibition against leukemia cancer cells with best results observed for compounds **17** and **22** exhibiting IC_{50} 1.8 and 2.0 μM respectively. As evident from various biological end-points, inhibition of cell proliferation by inducing G2/M cell cycle arrest, mitochondrial membrane disruption followed by DNA fragmentation and apoptosis is demonstrated.

1. Introduction

Natural products/molecules derived from plants have been a reliable source of therapeutic agents for the use in humans and the quest to swell these numbers goes unabated. Apart from the use of plant derived molecules directly as drugs such as vincristine, vinblastine, reserpine, etoposide, artemisinin; many plant derived scaffolds, through tailoring by the chemists, have resulted in the development of some of the most effective drugs e.g. khellin to sodium chromoglycate an anti-asthmatic drug, papaverine to verapamil for hypertension, galegine to metformin for diabetes. Therefore, the exploration of plants for their possible medicinal use needs to be continued. *Psoralea corylifolia* from the leguminosae family [1] is one of the medicinally important plants (in Ayurveda and Chinese System of Medicine) known for its inhibitory effect against dental caries [2], DNA polymerase and Topoisomerase II [3].

Recently, we carried out a study on bakuchiol (**1**) the major chemical constituent of *P. corylifolia*, wherein its antibacterial activity was enhanced through chemical modification to get MIC reduction up to eightfold against Gram +ve and Gram −ve bacteria [4]. Chemically, bakuchiol is made up of styryl moiety in conjunction with a monoterpene (together known as meroterpene). Compounds having the presence of styryl unit in conjunction with other moieties such as pyrone, chromone, quinazoline, in general, exhibit various biological activities such as anti HIV, anti-cancer etc. [5–8]. The fact that bakuchiol is reported to exhibit cytotoxicity against few human cancer cell lines [9], in particular against breast cancer [10], prompted us to carry out investigation on bakuchiol in a bid to have molecules with more potency, and possible clinical utility and application. In the present paper, the preparation of bakuchiol derivatives and their cytotoxicity study is described.

2. Results and discussion

Bakuchiol (**1**) is reported as cytotoxic toward breast cancer with IC_{50} 8.29×10^{-3} mol/L (MDA-MB-231) and 2.89×10^{-5} mol/L (T-47D) [11]. However, a detailed investigation of this molecule and its derivatives is still awaited. The bakuchiol derivatives (Fig. 1) prepared by modulation of phenolic group (**2**–**10**) or substitution in the aryl part (**11**–**13**, **28**), in the isopropylidene group (**15**–**16**), in the monoterpene part (**13**–**27**) and in the ethenyl group (**18**–**27**) [4], were tested at 50 μM concentration for their ability to induce cytotoxicity in human cancer cell lines encompassing lung (A-549), breast (MCF-7), prostate (PC-3), cervical (HeLa), leukemia (THP-1), CNS/neuroblastoma (IMR-32), and ovarian (OVCAR-5) cell lines (Table 1), taking mitomycin/adriamycin/5-FU as the gold standard.

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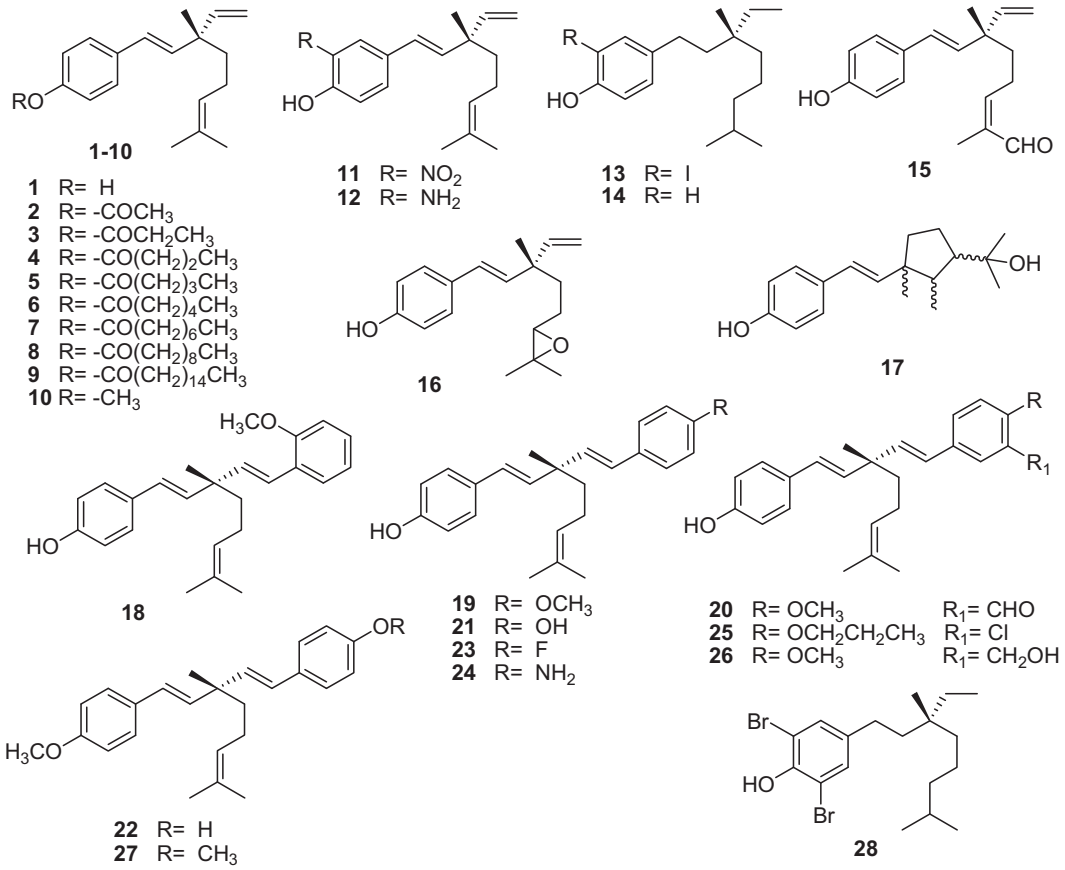

Fig. 1. Structures of bakuchiol **1** and its analogs **2**–**28**.

Table 1
Cytotoxic activity[a] (%age growth inhibition) of bakuchiol and its derivatives at 50 μM concentration against various human cancer cell lines.

Compounds	Breast MCF-7	Lung A-549	Prostate PC-3	Ovary OVCAR-5	CNS IMR-32	Cervical HeLa	Leukemia THP-1
1	**63 ± 1**	**63 ± 2**	**58 ± 1**	**66 ± 1**	**58 ± 3**	**55 ± 1**	**74 ± 2**
2	**75 ± 2**	**75 ± 3**	**67 ± 2**	**72 ± 1**	**74 ± 3**	**63 ± 1**	**82 ± 1**
3	23 ± 3	42 ± 1	54 ± 1	52 ± 1	63 ± 3	48 ± 3	60 ± 2
4	20 ± 2	50 ± 3	38 ± 3	41 ± 2	54 ± 3	36 ± 2	54 ± 2
5	25 ± 3	38 ± 2	42 ± 3	3 ± 1	21 ± 1	23 ± 2	15 ± 1
6	18 ± 3	58 ± 2	28 ± 2	13 ± 3	19 ± 1	50 ± 2	54 ± 2
7	12 ± 2	15 ± 3	22 ± 2	42 ± 3	51 ± 3	53 ± 3	62 ± 1
8	10 ± 1	48 ± 2	50 ± 3	42 ± 1	52 ± 2	50 ± 3	52 ± 1
9	24 ± 2	**61 ± 3**	**62 ± 3**	**64 ± 2**	**54 ± 1**	**51 ± 2**	**62 ± 2**
10	18 ± 3	12 ± 3	44 ± 2	37 ± 3	30 ± 2	43 ± 2	45 ± 3
11	**49 ± 2**	**52 ± 1**	**52 ± 3**	**55 ± 2**	**50 ± 3**	**49 ± 1**	**55 ± 3**
12	17 ± 4	41 ± 2	28 ± 1	33 ± 2	39 ± 3	48 ± 3	48 ± 3
13	40 ± 3	12 ± 2	08 ± 3	11 ± 3	21 ± 2	43 ± 3	58 ± 2
14	46 ± 2	49 ± 2	40 ± 2	43 ± 2	53 ± 2	42 ± 2	58 ± 2
15	12 ± 1	35 ± 3	51 ± 1	22 ± 3	53 ± 2	40 ± 3	54 ± 3
16	33 ± 3	44 ± 2	46 ± 3	12 ± 2	59 ± 3	32 ± 2	52 ± 3
17	**81 ± 2**	**76 ± 2**	**86 ± 2**	**75 ± 1**	**88 ± 3**	**68 ± 1**	**93 ± 2**
18	42 ± 3	10 ± 3	46 ± 1	38 ± 3	68 ± 1	50 ± 2	62 ± 2
19	55 ± 2	40 ± 2	50 ± 2	20 ± 2	6 ± 2	20 ± 3	52 ± 3
20	36 ± 3	32 ± 1	34 ± 3	33 ± 3	35 ± 3	31 ± 3	45 ± 3
21	31 ± 2	33 ± 1	32 ± 1	33 ± 2	30 ± 2	32 ± 3	66 ± 2
22	**79 ± 2**	**70 ± 2**	**93 ± 1**	**68 ± 1**	**69 ± 2**	**78 ± 2**	**88 ± 2**
23	28 ± 2	31 ± 1	33 ± 3	29 ± 2	37 ± 3	43 ± 1	60 ± 1
24	50 ± 2	57 ± 3	59 ± 2	16 ± 2	28 ± 2	28 ± 2	54 ± 3
25	47 ± 2	5 ± 3	7 ± 3	12 ± 1	8 ± 3	4 ± 3	20 ± 3
26	42 ± 2	41 ± 1	39 ± 2	38 ± 2	34 ± 2	36 ± 3	58 ± 2
27	13 ± 3	15 ± 1	29 ± 1	25 ± 2	27 ± 2	11 ± 1	62 ± 3
28	42 ± 1	40 ± 1	50 ± 2	08 ± 1	21 ± 2	42 ± 3	51 ± 3
5-Fu[b]	–	**78 ± 2**	**76 ± 1**	**75 ± 3**	**79 ± 2**	**71 ± 3**	**88 ± 3**
Mitomycin[b]	–	–	**70 ± 1**	–	–	–	–
Adriamycin[b]	**88 ± 3**	**76 ± 1**	–	–	–	–	–

The bold values are shown for those compounds which have proved to be active and those in normal font represent least significant.
[a] Results are mean ± SD of three separate experiments, conducted in triplicate at the concentration of 50 μM.
[b] Concentration of 5-FU = 20 μM, Mitomycin = 1 μM, Adriamycin = 1 μM.

Among the acyl derivatives (**2**–**9**), only analog **2** showed better growth inhibition (63–82%, bakuchiol 55–74%). Compound **17**, a 1,2,3-trisubstituted cyclopentane derivative obtained as an abnormal oxymercuration–demercuration reaction product of bakuchiol (characterized by spectral analysis), displayed excellent activity profile against leukemia and significant cytotoxicity against all other cell lines with 68–93% growth inhibition. Among bisstyryl derivatives (**18**–**27**), only compounds **18**, **19**, **22**, and **25** showed significant but different levels of cytotoxicity which were related to the nature and position of the substituent in the two aryl moieties. It is worth to mention here that a limited information is available related to the cytotoxicity of bisstyryl derivatives [11–13]. Interesting results were observed for two enantiomeric compounds **19** and **22** with *S*-isomer displaying far better activity than the *R*-isomer. While compound **19** (*R*-isomer) showed 50–55% growth inhibition only, its antipode (**22**) displayed 68–93% inhibitory effect against all the cancer cell lines across the board (Table 1), the exhibition of different levels of activity and toxicity associated with enantiomers is well known phenomena [14].

Further, the molecules that exhibited >50% inhibition (Table 1) were screened at lower concentration (30 μM) which resulted in the identification of **17**, **22**, and **2** as the most potent molecules (Table 2) and these were further tested for their inhibitory potential at lower concentrations (20, 10 and 5 μM). While compound **2** showed 57% growth inhibition at 20 μM against colon cancer cell line, **17** and **22** showed inhibition at 20 μM against all the cancer cell lines. Compound **17** displayed inhibition against four cancer cell lines and **22** against three cell lines at 10 μM. Both the compounds at 5 μM concentration, showed inhibition only against THP-1 cell line (Table 3).

The IC_{50} value for **2**, **17** and **22** was calculated by non-linear regression analysis using Graph Pad Software (2236 Avenida de la Playa La Jolla, CA 92037, USA). The derivatives displayed low IC_{50} values (<25–2.0 μM) against different cancer cell lines (Table 4).

Table 2
Cytotoxic activity[a] (%age growth inhibition) of bakuchiol and its selected derivatives at two different concentration against various human cancer cell lines.

Compounds	Conc. (μM)	Breast MCF-7	Liver Hep-2	Lung A-549	Prostate PC-3	Ovary OVCAR-5	CNS IMR-32	Cervical HeLa	Leukemia THP-1
1	50	**64 ± 1**	**53 ± 1**	**65 ± 2**	**59 ± 1**	**67 ± 1**	**61 ± 3**	**56 ± 1**	**76 ± 2**
	30	33 ± 1	20 ± 1	30 ± 2	21 ± 3	25 ± 3	31 ± 4	20 ± 1	44 ± 1
2	50	**77 ± 2**	**52 ± 3**	**72 ± 3**	**69 ± 2**	**71 ± 2**	**71 ± 3**	**65 ± 1**	**81 ± 1**
	30	**56 ± 3**	**51 ± 2**	**60 ± 2**	**60 ± 3**	**62 ± 2**	**59 ± 3**	45 ± 1	**63 ± 1**
8	50	26 ± 2	30 ± 1	**60 ± 1**	**60 ± 2**	**62 ± 2**	**53 ± 1**	**53 ± 2**	**64 ± 2**
	30	12 ± 1	16 ± 1	33 ± 1	27 ± 1	29 ± 2	22 ± 2	24 ± 2	39 ± 2
10	50	**51 ± 2**	40 ± 1	**53 ± 1**	**55 ± 3**	**57 ± 2**	**53 ± 2**	**50 ± 1**	**58 ± 3**
	30	37 ± 3	22 ± 1	34 ± 2	31 ± 1	29 ± 2	23 ± 2	28 ± 1	29 ± 2
17	50	**79 ± 2**	**65 ± 1**	**78 ± 2**	**88 ± 2**	**76 ± 1**	**85 ± 3**	**69 ± 1**	**95 ± 2**
	30	**58 ± 2**	**53 ± 1**	**61 ± 2**	**62 ± 2**	**55 ± 1**	**57 ± 2**	**52 ± 1**	**68 ± 1**
22	50	**77 ± 2**	**62 ± 1**	**72 ± 2**	**94 ± 1**	**67 ± 1**	**71 ± 2**	**80 ± 2**	**86 ± 2**
	30	**52 ± 2**	45 ± 1	**55 ± 3**	**50 ± 2**	**50 ± 1**	**51 ± 2**	**56 ± 2**	**60 ± 1**
24	50	48 ± 2	46 ± 1	**60 ± 3**	**61 ± 2**	18 ± 2	30 ± 2	27 ± 2	**51 ± 2**
	30	28 ± 3	32 ± 1	33 ± 2	15 ± 1	8 ± 2	14 ± 2	5 ± 2	32 ± 1
5-FU[b]	20	–	–	**76 ± 2**	**76 ± 1**	**78 ± 3**	**81 ± 2**	**74 ± 3**	**91 ± 3**
Mitomycin[b]	1	–	**81 ± 3**	–	–	–	–	–	–
Adriamycin[b]	1	**85 ± 3**	–	–	–	–	–	–	–

The bold values are shown for those compounds which have proved to be active and those in normal font represent least significant.
[a] Results are mean ± SD of three separate experiments, conducted in triplicate at the concentration of 50 μM.
[b] Concentration of 5-FU = 20 μM, Mitomycin = 1 μM, Adriamycin = 1 μM.

Table 3
Cytotoxic activity[a] (%age growth inhibition) of bakuchiol derivatives at 20 μM, 10 μM and 5 μM concentration against human cancer cell lines.

Compounds	Conc. (μM)	Breast MCF-7	Liver HEP-2	Lung A-549	Prostate DU-145	Leukemia THP-1	Prostrate PC-3	CNS IMR-32	Colon HCT-15
2	20	44 ± 2	46 ± 2	1 ± 2	42 ± 2	50 ± 2	45 ± 3	48 ± 3	**57 ± 2**
	10	31 ± 2	30 ± 1	22 ± 1	29 ± 2	44 ± 2	32 ± 1	34 ± 2	46 ± 1
	5	15 ± 2	14 ± 3	13 ± 2	19 ± 1	34 ± 1	21 ± 2	27 ± 2	31 ± 2
17	20	**50 ± 2**	**50 ± 1**	**53 ± 3**	**54 ± 2**	**76 ± 2**	**57 ± 2**	**62 ± 2**	**63 ± 3**
	10	32 ± 1	34 ± 3	40 ± 2	**50 ± 1**	**65 ± 3**	35 ± 2	**50 ± 1**	**55 ± 3**
	5	27 ± 2	17 ± 2	32 ± 3	32 ± 3	**54 ± 2**	24 ± 4	46 ± 1	29 ± 3
22	20	**50 ± 3**	**58 ± 4**	**69 ± 1**	**73 ± 2**	**78 ± 4**	**58 ± 2**	40 ± 3	**61 ± 3**
	10	37 ± 1	46 ± 1	47 ± 1	**57 ± 1**	**62 ± 1**	41 ± 1	32 ± 1	**50 ± 2**
	5	24 ± 1	23 ± 1	24 ± 1	20 ± 4	**53 ± 2**	23 ± 2	13 ± 1	28 ± 3
5-Fu[b]	20	–	–	**78 ± 2**	**75 ± 1**	**85 ± 3**	**75 ± 3**	**83 ± 2**	**63 ± 1**
Mitomycin[b]	1	–	**78 ± 3**	–	–	–	–	–	–
Adriyamycin[b]	1	**91 ± 1**	–	–	–	–	–	–	–

The bold values are shown for those compounds which have proved to be active and those in normal font represent least significant.
[a] Results are mean ± SD of three separate experiments, conducted in triplicate at the concentration of 50 μM.
[b] Concentration of 5-FU = 20 μM, Mitomycin = 1 μM, Adriamycin = 1 μM.

Table 4
IC_{50} values of selected compounds **2**, **17**, **22** against various human cancer cell lines.

Tissue type	Leukemia		Prostate		Liver	Breast	Lung	CNS	Colon
Cell lines	THP-1	HL-60	DU-145	PC-3	HEP-2	MCF-7	A-549	SF-295	HCT-15
Compounds	IC_{50} in μM								
17	3.5 ± 1.1	1.8 ± 1.2	14 ± 2	19 ± 1	19 ± 2	22 ± 3	17 ± 3	23 ± 2	13 ± 1
22	4.2 ± 1.3	2 ± 1.1	11 ± 3	15 ± 3	14 ± 2	23 ± 3	12 ± 1	22 ± 3	13 ± 1
2	18 ± 2	ND	26 ± 3	23 ± 2	20 ± 1	25 ± 2	40 ± 3	8 ± 1	11 ± 2

ND = not determined.

Since leukemic cell lines were found the most sensitive cells toward the cytotoxic potential of these compounds, the IC_{50} value was further calculated at two time points (48 h and 24 h) on HL-60 cells by MTT assay. Both compounds **17** and **22** showed concentration and time dependent inhibition of cell proliferation displaying the IC_{50} values 1.8 μM and 18 μM for **17** while 2.0 μM and 16 μM for **22** after 48 h and 24 h time incubation respectively (Figs. 2 and 3).

Further experiments were carried out to verify whether the cancer cell death induced by the **17** and **22** was apoptotic, as it became increasingly evident that although the primary intracellular targets and the pharmacological mechanisms of action of the anti-cancer drugs vary vastly, the drug induced cell killing is generally mediated by apoptosis [15]. Compounds **17** and **22** were observed to be potent apoptosis inducers, as evidenced from the measurement of two important biological end-points of the apoptosis viz., DNA fragmentation and increase in sub-G0 DNA fraction. The apoptotic potential of **17** and **22** was confirmed through induction of DNA fragmentation in HL-60 cells, which is known as the hallmark of apoptosis. Compound **17** induced the laddering pattern of apoptosis at a concentration of 20 μM, and in **22**, the laddering pattern was concentration dependent. The minimal concentration inducing DNA fragmentation of 20 μM, which on extension to 40 μM showed no smear formation which is a representative of post apoptotic necrosis (Figs. 4 and 5).

Further extending our study, DNA cell cycle analysis was performed using HL-60 cells. Most of the differentiated cells are arrested in the G1 phase but in case of cancer cells, this control is lost and they go on dividing. Thus, **17** and **22** were subjected to hypo-diploid sub-G0 DNA fraction (<2nDNA) analysis as a measure of apoptosis. HL-60 cells treated with **17** and **22** at 30 μM concentration showed a considerable increase in the hypo-diploid sub-G0 DNA fraction (<2nDNA), i.e. 68% in case of **17** and 66% in case of **22** at 24 h treatment, indicating DNA damage (Figs. 6 and 7).

To rule out whether the compounds have any effect on the mitochondrial functioning, mitochondrial membrane depolarization assay was performed, both **17** and **22** caused the disruption of mitochondrial membrane and subsequent loss of mitochondrial membrane potential in a concentration dependent manner. Compound **17** showed almost 54% of dissipation of mitochondrial membrane potential and **22** showed 55.4%, the untreated control showed only 6% and cells treated with camptothecin showed 55.5% (at 5 μM) suggesting the central role of mitochondria toward the apoptotic potential of both of these molecules (Figs. 8 and 9).

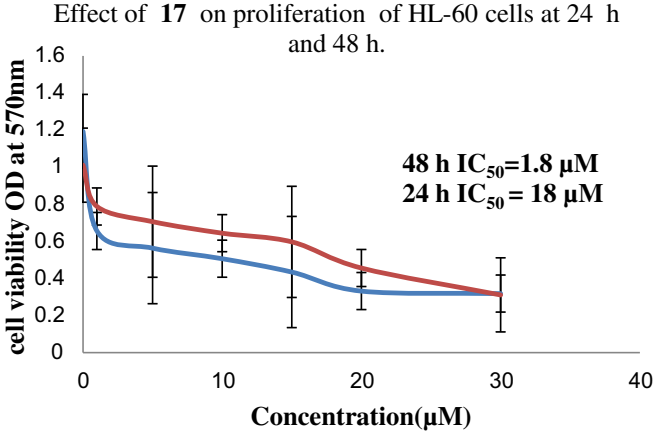

Fig. 2. Effect of **17** on cell proliferation of human leukemia cell line (HL-60). Exponentially growing HL-60 cells (15×10^3) were seeded in 96-well plate. DMSO solution of **17** was added to the cells at different conc. whereas the untreated control received the vehicle only. MTT dye was added after 44 h and the plates were incubated for the next 4 h at 37 °C and the OD was measured as described in the experimental section. Data are mean ± SD ($n = 6$ wells) and representative of two similar experiments.

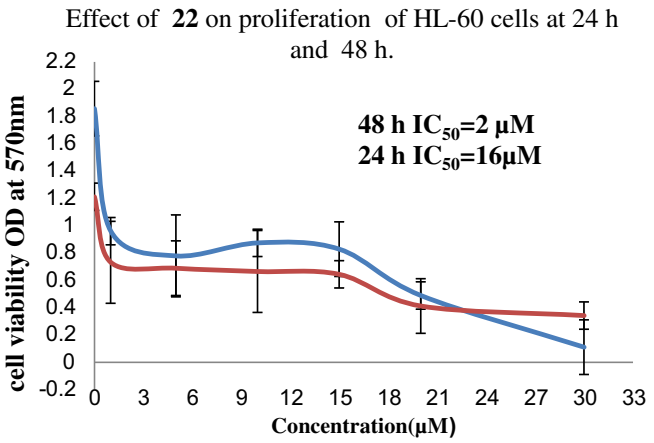

Fig. 3. Effect of **22** on cell proliferation of human leukemia cell line (HL-60). Exponentially growing HL-60 cells ($15 \times 10^{3)}$ were seeded in 96-well plate. DMSO solution of **22** was added to the cells at different conc. whereas the untreated control received the vehicle only. MTT dye was added after 44 h and the plates were incubated for the next 4 h at 37 °C and the OD was measured as described in the experimental section are mean ± SD ($n = 6$ wells) and representative of two similar experiments.

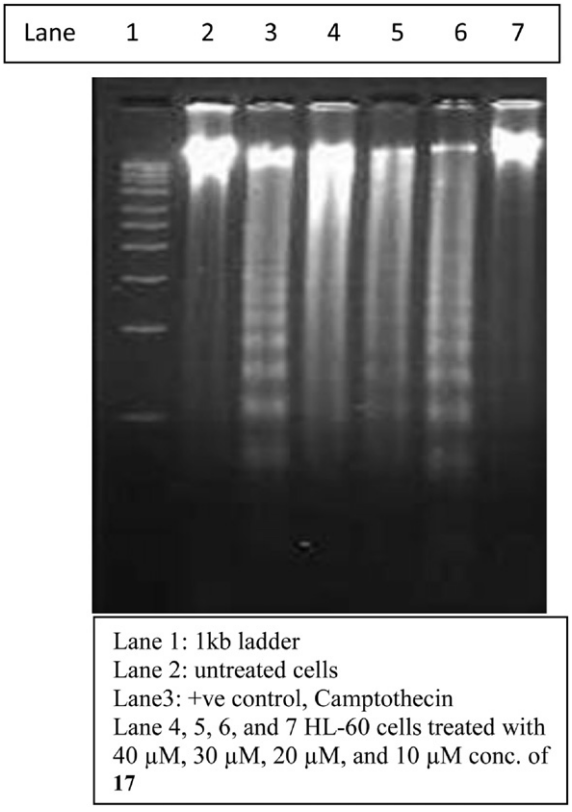

Fig. 4. Compound **17** induced DNA fragmentation in HL-60 cells. Cells 2×10^6/mL/well were treated with indicated conc. of **17** for 24 h. Genomic DNA was isolated and put to electrophoresis as described in experimental section to assess the fragmentation at different concentrations.

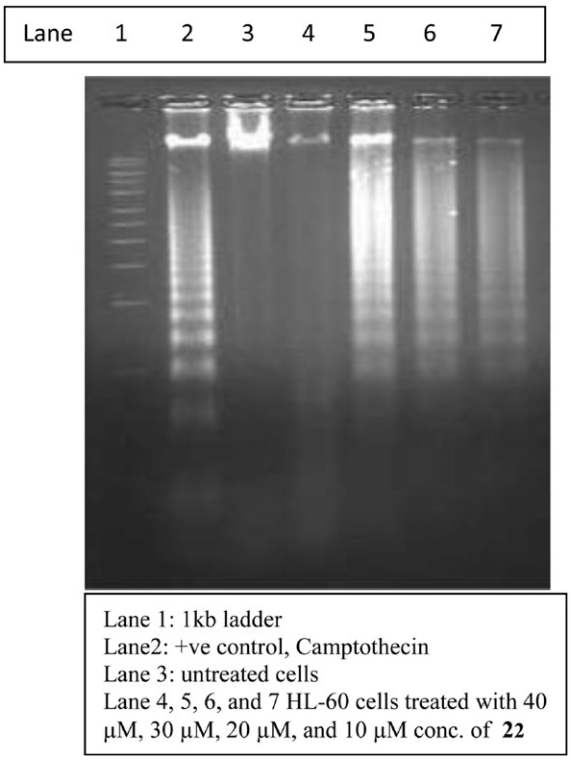

Fig. 5. Compound **22** induced DNA fragmentation in HL-60 cells. Cells 2×10^6/mL/well were treated with indicated conc. of **22** for 24 h. Genomic DNA was isolated and put to electrophoresis as described in the experimental section to assess the fragmentation at different concentrations.

3. Conclusion

We demonstrated the preparation of diversified analogs such as acyl, nitro, amino, halo, alkyloxy, epoxy, formyl, and styryl derivatives, of the natural product bakuchiol and their ability to inhibit several cancer cell lines (IC_{50} 1.8 to <25 μM). Our data revealed that compounds **17** and **22** provide considerable evidence for their apoptotic mode of action against various cancer cell lines especially HL-60. Moreover, the induction of apoptosis by these molecules was associated with perturbance in mitochondrial membrane potential and cell cycle arrest that ultimately resulted in appreciable DNA fragmentation. However, kinetic and *in vivo* studies in the preclinical models need to be explored in order to ascertain their therapeutic potency.

4. Experimental

4.1. Chemistry

All reagents used for chemical synthesis were purchased from Sigma–Aldrich. Solvents were distilled before use. All the reactions were monitored by TLC on silica gel F_{254} plates (E. Merck) using 2% ceric ammonium sulfate solution for detection of the spots. Flash chromatography was carried out for purification of the products. All NMR spectra were recorded on Bruker DPX 200, DPX 400 and DPX 500 instruments using $CDCl_3$ as the solvent with TMS as internal standard. The chemical shifts are expressed in delta whereas coupling constants in Hertz. Mass spectra were recorded on ESI-esquire 3000 Bruker Daltonics instrument. IR recorded on FT Bruker (270-30) spectrophotometer. The purity of all the compounds (bakuchiol and its analogs) was determined by GC–MS/HPLC and found in the range 93–98%. HPLC/GCMS chromatograms of some of the analogs are provided in Supporting information.

4.1.1. Isolation of bakuchiol

Bakuchiol was isolated in bulk quantity from the seeds of *P. corylifolia* (duly authenticated by the Taxonomist of our institute). Bakuchiol used in the present study was of >98% purity achieved by repeated column chromatography over silica gel and the natural product was well characterized by spectroscopic study (found in agreement with the literature data) [16].

4.1.2. General procedure for preparation of acyl derivatives of bakuchiol (**2**–**9**)

Compounds **2**–**9** were synthesized by treating bakuchiol **1** with appropriate alkanoic anhydride in presence of catalytic amount of *N*,*N*-dimethyl amino pyridine and the progress of reaction monitored by TLC. On completion of the reaction, the contents poured in ice water and the oily material separated and dissolved in DCM. The organic layer washed with water, dried over anhydrous calcium chloride and concentrated under reduced pressure. The acyl derivatives thus obtained were purified by column chromatography (CC) over silica gel 60–120 mesh, using hexane:Ethyl acetate mixture (49:1) as eluent to give compounds **2**–**9** in >90% overall yield. The spectral data of all the derivatives of **1** are given below.

*4.1.2.1. Synthesis of 4-(3,7-dimethyl-3-vinylocta-1,6-dienyl) phenyl acetate (**2**).* The title compound was prepared and purified by the procedure as described above to give **2** in 98% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.20 (3H, s, $-C(CH{=}CH_2)CH_3$), 1.48–1.50 (2H, m, $-C(CH_3)CH_2-$), 1.57 and 1.67 (3H each, s, $=C(CH_3)_2$), 1.89–2.04 (2H, m, $-CH_2CH_2CH{=}$), 2.28 (3H, s, $-OCOCH_3$), 5.05 (3H, m, $-CH_2CH{=}$ and $-C(CH{=}CH_2)$), 5.87 (1H, dd, $J = 17.2$ and 10.87 Hz, $-C(CH{=}CH_2)$), 6.14 (1H, d, $J = 16.2$ Hz, Ar–CH=CH–), 6.30 (1H, d, $J = 16.2$ Hz, Ar–CH=), 7.01 (2H, d, $J = 8.58$ Hz, 2× Ar–H), 7.35 (2H, d, $J = 8.58$ Hz,2× Ar–H). ^{13}C NMR (125 MHz): δ 17.68, 21.14, 23.26, 23.34, 25.73, 41.26, 42.68, 112.17, 121.59, 124.75, 126.33, 127.01, 131.38, 135.76, 138.24, 145.69, 149.62, 169.52. IR: 668, 757, 1019, 1084, 1160, 1216, 1403, 1457, 1513, 1541, 1635, 1765, 2853, 2924, 3421 cm^{-1}. MS *m*/*z* 321 ($M^+ + Na$).

*4.1.2.2. Synthesis of 4-(3,7-dimethyl-3-vinylocta-1,6-dienyl) phenyl propanoate (**3**).* The title compound was prepared and purified by the procedure as described under Section 4.1.2 to afford **3** in 97% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.20, (3H, s, $-C(CH{=}CH_2)CH_3$), 1.25 (3H, t, $J = 7.3$ Hz, $-CH_2CH_3$), 1.49 (2H, m, $-C(CH_3)CH_2-$), 1.58 and 1.68, (3H each, s, $=C(CH_3)_2$), 1.94 (2H, m, $-CH_2CH_2CH{=}$), 2.54 (2H, q, $J = 15.1$ and 7.27 Hz, $-COCH_2-$), 4.97–5.01 (3H, m, $CH_2CH{=}$ and $-C(CH{=}CH_2)$), 5.88 (1H, dd, $J = 17.24$, 10.90 Hz, $-C(CH{=}CH_2)$), 6.15 (1H d, $J = 16.27$ Hz, Ar–CH=CH–), 6.31 (1H, d, $J = 16.28$ Hz, Ar–CH=), 7.00 (2H, d, $J = 8.57$ Hz, 2× Ar–H), 7.36 (2H, d, $J = 8.58$ Hz, 2× Ar–H). ^{13}C NMR (125 MHz): δ 10.18, 17.63, 22.83, 23.36, 25.73, 27.76, 41.37, 42.75, 112.15, 121.73, 124.86, 126.43, 127.02, 131.23, 135.66, 138.16, 145.73, 149.87, 172.24. IR: 665, 754, 1021, 1088, 1161, 1215, 1401, 1457, 1515, 1635, 1762, 2853, 2924, 3421 cm^{-1}. MS *m*/*z* 335 ($M^+ + Na$).

*4.1.2.3. Synthesis of 4-(3,7-dimethyl-3-vinylocta-1,6-dienyl) phenyl butyrate (**4**).* The title compound was prepared and purified by the procedure as described under Section 4.1.2 to afford **4** in 97% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.96 (3H, t, $J = 7.38$ Hz, $-CH_2CH_3$), 1.19 (3H, s, $-C(CH{=}CH_2)CH_3$), 1.46–1.53, (2H, m, $-C(CH_3)CH_2-$), 1.57 and 1.67 (3H each, s, $=C(CH_3)_2$), 1.74–1.98, (4H, m, $-CH_2CH_2CH{=}$, $-CH_2CH_3$), 2.53 (2H, t, $J = 7.35$ Hz, $-COCH_2-$), 4.97–5.01 (3H, m, $-CH_2CH{=}$ and $-C(CH{=}CH_2)$), 5.88 (1H, dd,

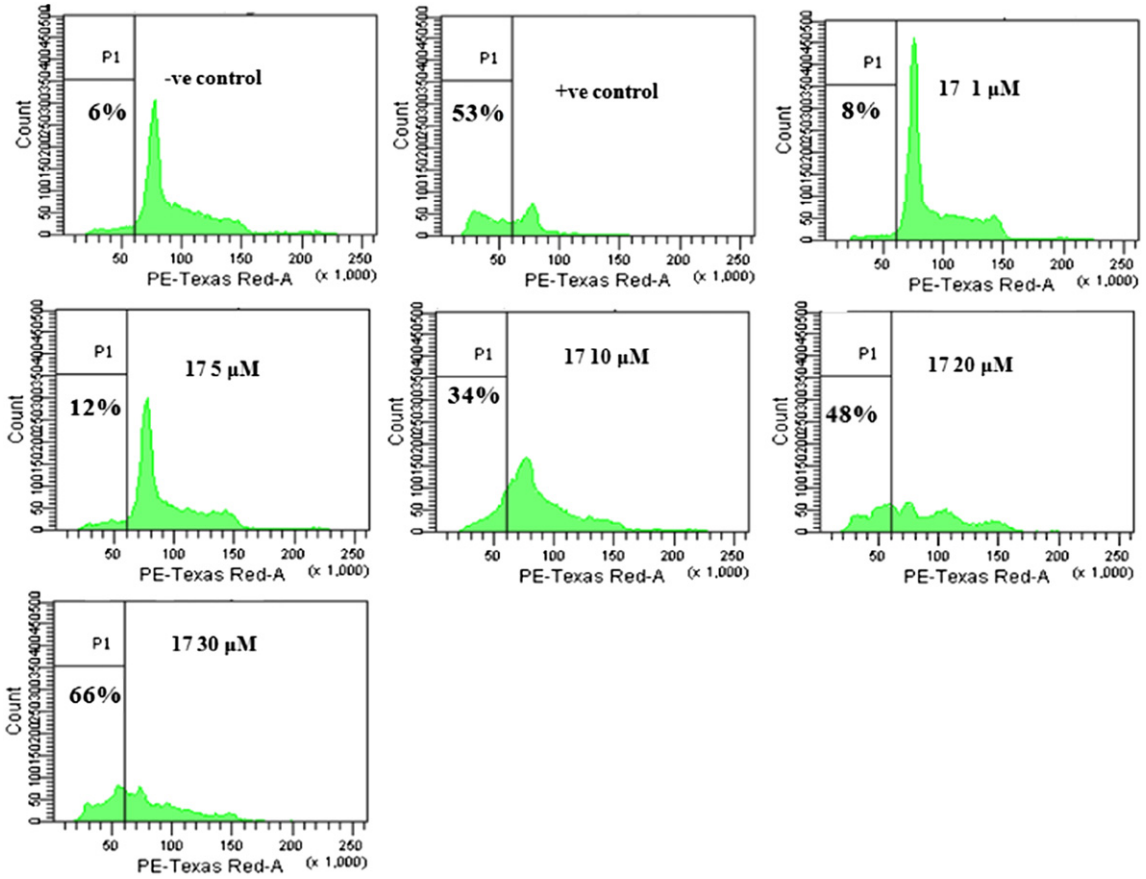

Fig. 6. HL-60 cells (1×10^6/mL) in culture were treated with indicated conc. of **17** for 24 h. Cells were stained with PI to determine DNA fluorescence and cell cycle phase distribution by flow cytometry. Fraction of cells for hypo-diploid (sub-G0, ≤2n DNA) population indicative of DNA damage was analyzed and shown as (%). Data are representative of one of two similar experiments.

$J = 17.29$, 10.86 Hz, −C(C*H*═CH_2)), 6.15 (1H, d, $J = 16.25$ Hz, Ar−CH═C*H*−), 6.31 (1H, d, $J = 16.24$ Hz, Ar−C*H*═), 7.01 (2H, d, $J = 8.43$ Hz, 2× Ar−*H*), 7.35 (2H, d, $J = 8.44$ Hz, 2× Ar−*H*). ^{13}C NMR (125 MHz): δ 13.65, 17.64, 18.46, 23.21, 23.26, 25.70, 36.23, 41.20, 42.64, 112.11, 121.57, 124.69, 126.30, 126.94, 131.38, 135.58, 138.10, 145.66, 149.62, 172.20. IR: 750, 1025, 1083, 1160, 1214, 1403, 1458, 1512, 1630, 1760, 2850, 2921, 3422 cm^{-1}. MS at *m*/*z* 349 ($M^+ + Na$).

*4.1.2.4. Synthesis of 4-(3,7-dimethyl-3-vinylocta-1,6-dienyl) phenyl pentanoate (**5**).* The title compound was prepared and purified by the procedure as described under Section 4.1.2 to afford **5** in 97% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, $J = 7.3$ Hz, −CH_2CH_3), 1.12 (3H, s, −C(CH═CH_2)C*H*$_3$), 1.26–1.53 (4H, m, −C(CH_3)C*H*$_2$−, −C*H*$_2$$CH_2CH_3$), 1.58 and 1.67 (3H each, s, ═C(C*H*$_3$)$_2$), 1.76 (2H, m, −C*H*$_2$$CH_3$), 1.95 (2H, m, −$CH_2$C*H*$_2$CH═), 2.53 (1H, t, $J = 7.57$ Hz, −COC*H*$_2$−), 4.96 (2H, dd, $J = 18.99$, 11.33 Hz, −C(CH═C*H*$_2$)), 5.10 (1H, t, $J = 7.02$ Hz, −CH_2C*H*═), 5.87 (1H, dd, $J = 17.43$, 10.70 Hz, −C(C*H*═CH_2)), 6.15 (1H, d, $J = 16.25$ Hz, Ar−CH═C*H*−), 6.30 (1H, d, $J = 16.23$ Hz, Ar−C*H*═), 7.00 (2H, d, $J = 8.48$ Hz, 2× Ar−*H*), 7.35 (2H, d, $J = 8.49$ Hz, 2× Ar−*H*). ^{13}C NMR (125 MHz): δ 13.69, 17.59, 22.22, 23.21, 23.27, 25.65, 27.19, 34.07, 41.21, 42.61, 112.09, 121.53, 124.71, 126.32, 126.91, 131.26, 135.56, 138.07, 145.63, 149.65, 172.31. IR: 751, 1024, 1085, 1158, 1212, 1401, 1448, 1510, 1628, 1761, 2851, 2919, 3425 cm^{-1}. MS at *m*/*z* 363 ($M^+ + Na$).

*4.1.2.5. Synthesis of 4-(3,7-dimethyl-3-vinylocta-1,6-dienyl) phenyl hexanoate (**6**).* The title compound was prepared and purified by the procedure as described under Section 4.1.2 to afford **6** in 95% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.91 (3H, br s, −CH_2C*H*$_3$), 1.20 (s, 3H, −C(CH═CH_2)C*H*$_3$), 1.26–1.53 (6H, m, −C(CH_3)C*H*$_2$−, −(C*H*$_2$)$_2$$CH_2CH_3$), 1.58 and 1.67 (3H each, s, ═C(C*H*$_3$)$_2$), 1.75–1.97, (4H m, −C*H*$_2$C*H*$_3$, −CH_2C*H*$_2$CH═), 2.53 (2H, t, $J = 7.27$ Hz, −COC*H*$_2$−), 4.97–5.01 (3H, m, C(CH═C*H*$_2$), −CH_2C*H*═), 5.87 (1H, dd, $J = 17.22$, 10.90 Hz, −C(C*H*═CH_2)), 6.15 (1H, d, $J = 16.24$ Hz, Ar−CH═C*H*−), 6.31 (1H, d, $J = 16.24$ Hz, Ar−C*H*═), 7.00 (2H, d, $J = 8.42$ Hz, 2× Ar−*H*), 7.35 (2H, d, $J = 8.40$ Hz, 2× Ar−*H*). ^{13}C NMR (125 MHz): δ 14.15, 17.65, 22.72, 23.57, 24.64, 25.69, 29.39, 31.29, 34.36, 41.25, 42.75, 112.12, 121.56, 124.75, 126.36, 126.93, 131.26, 135.58, 138.08, 145.66, 149.72, 172.23. IR: 748, 1021, 1084, 1156, 1202, 1405, 1445, 1513, 1622, 1762, 2852, 2921, 3423 cm^{-1}. MS at *m*/*z* 377 ($M^+ + Na$).

*4.1.2.6. Synthesis of 4-(3,7-dimethyl-3-vinylocta-1,6-dienyl) phenyl octanoate (**7**).* The title compound was prepared and purified by the procedure as described under Section 4.1.2 to afford **7** in 95% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89 (3H, br s, −CH_2C*H*$_3$), 1.20 (3H, s, −C(CH═CH_2)C*H*$_3$), 1.30 (4H, br s, 2× C*H*$_2$), 1.45 (6H, m, 3× C*H*$_2$), 1.58 and 1.67 (3H each, s, ═C(C*H*$_3$)$_2$), 1.79–1.97 (4H, m, −C*H*$_2$$CH_3$, −$CH_2$C*H*$_2$CH═), 2.53 (2H, t, $J = 7.39$ Hz, −COC*H*$_2$−), 4.97–5.10 (3H, m, C(CH═C*H*$_2$), −CH_2C*H*═), 5.88 (1H, dd, $J = 10.89$, 17.24 Hz, −C(C*H*═CH_2)), 6.15 (1H, d, $J = 16.27$ Hz, Ar−CH═C*H*−), 6.31 (1H, d, $J = 16.28$ Hz, Ar−C*H*═), 6.96 (2H, d, $J = 8.23$ Hz, 2× Ar−*H*), 7.36 (2H, d, $J = 8.34$ Hz, 2× Ar−*H*). ^{13}C NMR (125 MHz): δ 14.08, 17.68, 22.62, 23.28, 24.94, 25.70, 28.95, 29.09, 29.36, 31.95, 34.36, 41.23, 42.63, 112.12, 121.55, 124.74, 126.35, 127.90, 131.23, 135.33, 138.02, 145.61, 149.69, 172.20. IR: 750, 1020, 1080, 1155,

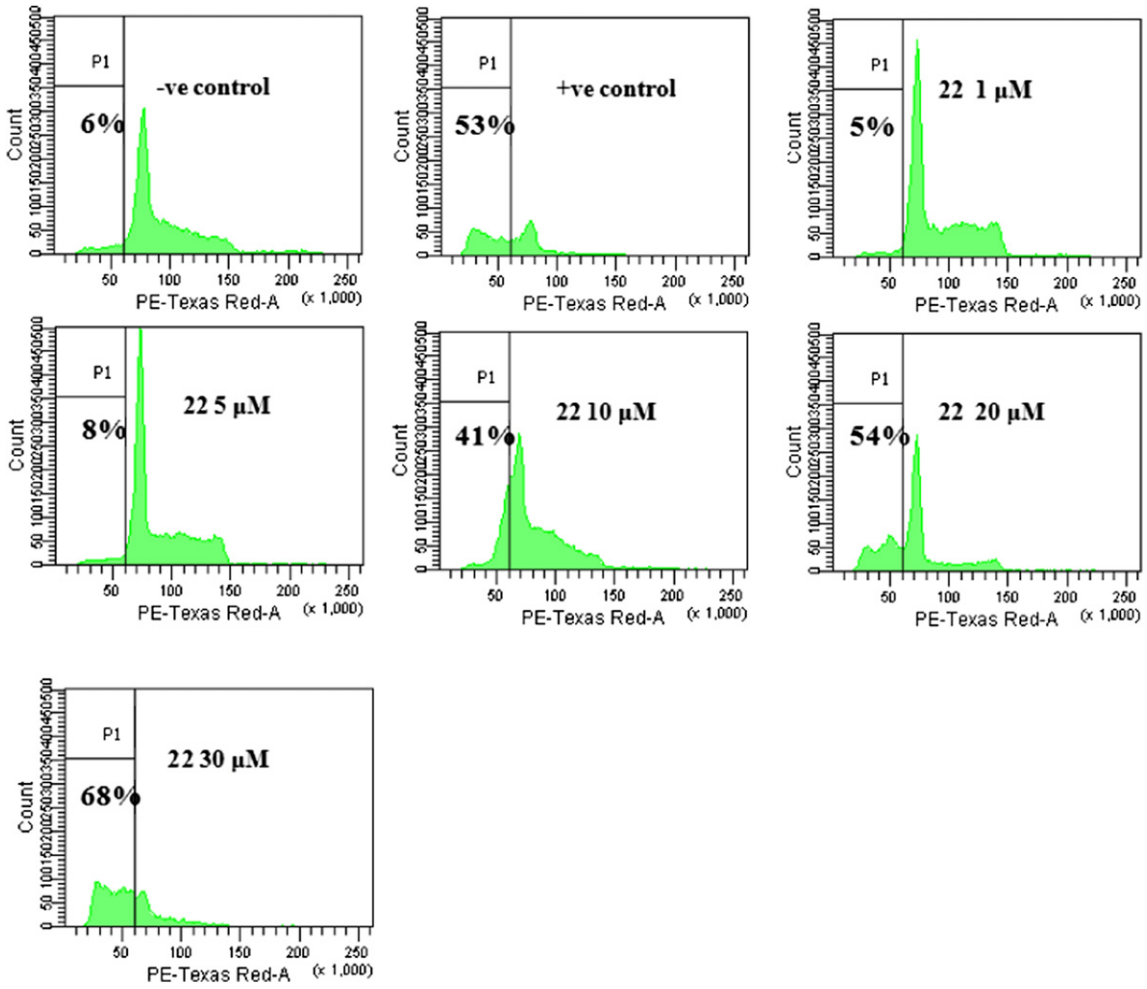

Fig. 7. HL-60 cells (1×10^6/mL) in culture were treated with indicated conc. of **22** for 24 h time period. Cells were stained with PI to determine DNA fluorescence and cell cycle phase distribution by flow cytometry. Fraction of cells for hypo-diploid (sub-G0, ≤2n DNA) population indicative of DNA damage was analyzed and shown as (%). Data are representative of one of two similar experiments.

1215, 1402, 1449, 1512, 1625, 1763, 2853, 2918, 3426 cm^{-1}. MS at *m/z* 405 ($M^+ + Na$).

*4.1.2.7. Synthesis of 4-(3,7-dimethyl-3-vinylocta-1,6-dienyl) phenyl decanoate (**8**).* The title compound was prepared and purified by the procedure as described under Section 4.1.2 to afford **8** in 93% yield. ^1H NMR (200 MHz, $CDCl_3$): δ 0.83 (3H, br s, $-CH_2CH_3$), 1.20 (3H, s, $-C(CH{=}CH_2)CH_3$), 1.25 (8H, br s, 4× CH_2), 1.46 (6H, m, 3× CH_2), 1.58 and 1.67 (3H each, s, $=C(CH_3)_2$), 1.97 (4H, m, $-CH_2CH_3$, $-CH_2CH_2CH{=}$), 2.53 (2H, t, $J = 7.39$ Hz, $-COCH_2-$), 4.93–5.10 (3H, m, $-C(CH{=}CH_2)$, $-CH_2CH{=}$), 5.87 (1H, dd, $J = 10.93$, 17.28 Hz, $-C(CH{=}CH_2)$), 6.07 (1H, d, $J = 15.97$ Hz, Ar–CH=CH–), 6.23 (1H, d, $J = 15.97$ Hz, Ar–CH=), 7.01 (2H, d, $J = 8.3$ Hz, 2× Ar–H), 7.35 (2H, d, $J = 8.38$ Hz, 2× Ar–H). ^{13}C NMR (125 MHz): δ 14.18, 17.63, 22.83, 23.30, 25.05, 25.73, 29.25, 29.50, 29.59, 29.73, 29.88, 32.0, 34.43, 41.37, 42.74, 112.15, 121.70, 124.86, 126.43, 127.01, 131.23, 135.66, 138.16, 145.73, 149.87, 172.24. IR: 753, 1022, 1081, 1153, 1214, 1405, 1447, 1513, 1628, 1760, 2855, 2919, 3423 cm^{-1}. MS at *m/z* 433 ($M^+ + Na$).

*4.1.2.8. Synthesis of 4-(3,7-dimethyl-3-vinylocta-1,6-dienyl) phenyl palmitate (**9**).* The title compound was prepared and purified by the procedure as described under Section 4.1.2 to afford **9** in 92% yield. ^1H NMR (200 MHz, $CDCl_3$): δ 0.90 (3H, br s, $-CH_2CH_3$), 1.20 (3H,s, $-C(CH{=}CH_2)CH_3$), 1.26 (20H, br s, 10× CH_2), 1.46–1.53 (6H, 3× CH_2), 1.57 and 1.67 (2H each, s, $=C(CH_3)_2$), 1.73–1.98 (4H, m, $-CH_2CH_3$, $-CH_2CH_2CH{=}$), 2.53 (2H, t, $J = 7.28$ Hz, $-COCH_2-$), 4.97–5.01 (3H, $-C(CH{=}CH_2)$, $-CH_2CH{=}$), 5.88 (1H, dd, $J = 17.13$, 10.92 Hz, $-C(CH{=}CH_2)$), 6.15 (1H, d, $J = 16.25$ Hz, Ar–CH=CH–), 6.31 (1H, d, $J = 16.24$ Hz, Ar–CH=), 7.00 (2H, d, $J = 8.46$ Hz, 2× Ar–H), 7.35 (2H, d, $J = 8.47$ Hz, 2× Ar–H). ^{13}C NMR (125 MHz): δ 14.14, 17.63, 22.72, 23.23, 23.28, 24.96, 25.70, 29.13, 29.28, 29.40, 29.49, 29.62, 29.72, 31.95, 34.40, 41.23, 42.64, 112.12, 121.55, 124.72, 126.34, 126.93, 131.29, 135.56, 138.06, 145.63, 149.67, 172.32. IR: 750, 1020, 1080, 1155, 1215, 1402, 1449, 1512, 1625, 1763, 2853, 2918, 3426 cm^{-1}. MS at *m/z* 517 ($M^+ + Na$).

*4.1.2.9. Synthesis of 1-methoxy-4-(3,7-dimethyl-3-vinylocta-1,6-dienyl)benzene (**10**).* Bakuchiol (2 mmol) was dissolved in acetone (5 mL) and anhydrous K_2CO_3 (3 mmol) was added followed by 2 mmol of methyl iodide. The mixture was allowed to reflux for 8 h. The reaction mixture was cooled, filtered, and evaporated. The product was purified by flash chromatography using hexane:EtOAc (19:1) as the eluent to afford **10** in 93% yield. ^1H NMR (500 MHz, $CDCl_3$): δ 1.21 (3H, s, $-C(CH{=}CH_2)CH_3$), 1.51 (2H, m, $-C(CH_3)CH_2CH_2-$), 1.59 and 1.69 (3H each, s, $=C(CH_3)_2$), 1.96 (2H, m, $-CH_2CH{=}$), 3.80 (3H, s, $-OCH_3$), 5.03 (2H, m, $-C(CH{=}CH_2)CH_3$), 5.12 (1H, t, $J = 7.28$ Hz, $-CH{=}C$), 5.89 (1H, dd, $J = 10.95$, 17.18

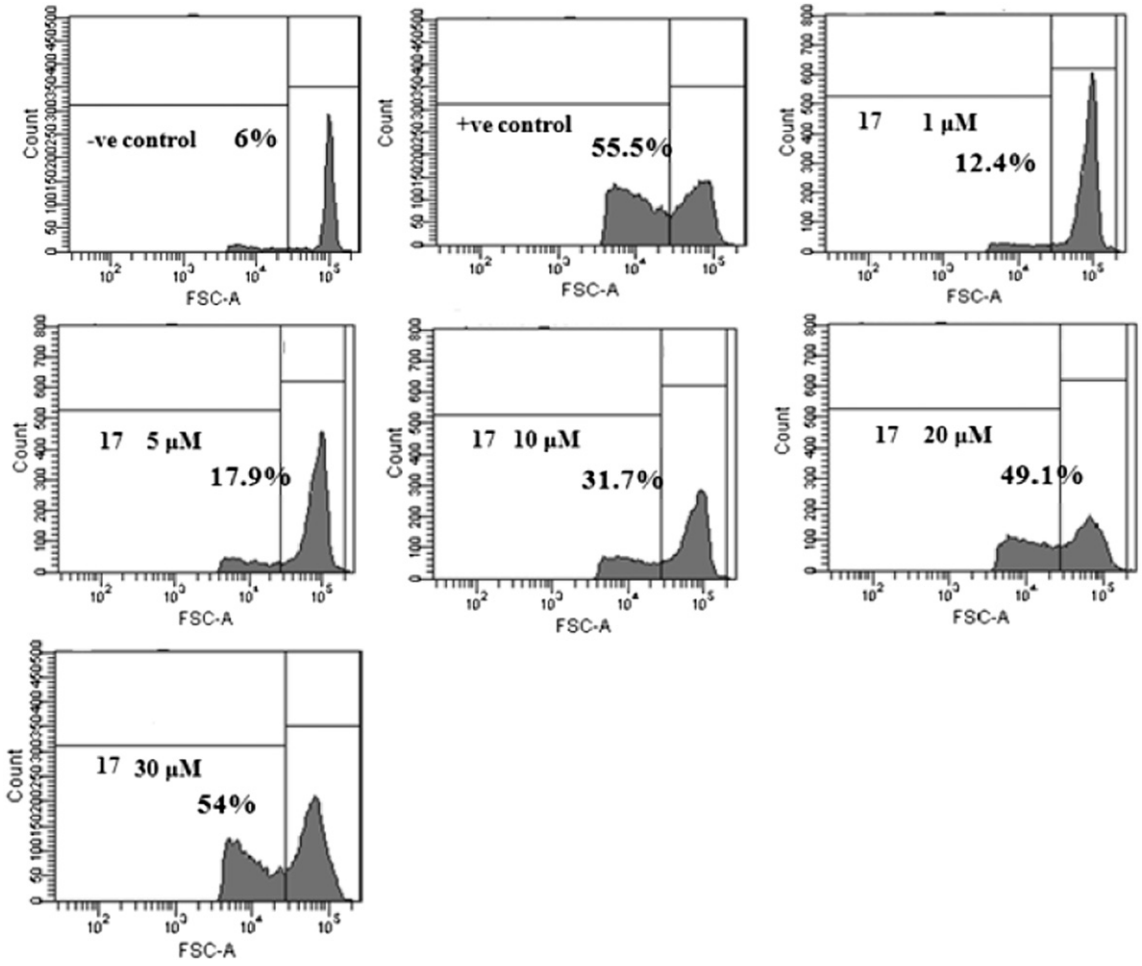

Fig. 8. Compound **17** induced loss of mitochondrial membrane potential ($\Delta\Psi_m$) HL-60. Cells (1×10^6/mL/ well) incubated with **17** at different conc. in 6 well plates for 24 h. Before 1 h of the completion of the experiment cells were treated with Rodamine-123 (5 μM). Cells were washed with PBS and centrifuged and finally dissolved in 1 mL of PBS and analyzed. Data are representative of one of two similar experiments.

Hz,−C(C*H*═CH$_2$)), 6.08 (1H, d, J = 16.18 Hz, Ar−CH═C*H*−), 6.28 (1H, d, J = 16.18 Hz, Ar−C*H*═CH−), 6.85 (2H, d, J = 8.21 Hz, 2× Ar−*H*), 7.31 (2H, d, J = 8.21 Hz, 2× Ar−*H*). ^{13}C NMR (125 MHz): δ 19.09, 24.70, 24.83, 27.15, 42.77, 43.98, 56.73, 113.3, 115.3, 126.29, 128.01, 128.61, 132.16, 132.71, 137.26, 147.45, 160.21. IR: 814, 912, 970, 1037, 1174, 1248, 1462, 1510, 1608, 1632, 2854, 2915, 2965, 3384 cm^{-1}. MS at m/z 293 ($M^+ + Na$).

*4.1.2.10. Synthesis of 3-nitro,4-(3,7-dimethyl-3-vinylocta-1,6-dienyl) phenol (**11**).* To a stirred solution of the bakuchiol (1 mmol) in acetone (15 mL) was added nickel (II) nitrate (1 mmol) followed by a catalytic amount of *p*-TSA (0.012 mmol) and the reaction mixture was refluxed until **1** was fully consumed. Acetone was removed under vacuum and the crude mass was partitioned between DCM and water. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified by CC over silica gel using hexane:EtOAc (49:1) as the eluent to furnish **11** in 86% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.22 (3H, s, −C(CH═CH$_2$)C*H*$_3$), 1.51 (2H, m, −C(CH$_3$)C*H*$_2$CH$_2$−), 1.59 and 1.68 (3H each, s, ═C(C*H*$_3$)$_2$), 1.96 (2H, m, −C*H*$_2$CH═), 5.06 (3H, m, −C(CH═C*H*$_2$)CH$_3$, −CH$_2$C*H*═C), 5.88 (1H, dd, J = 10.92, 17.22 Hz, −C(C*H*═CH$_2$)), 6.17 (1H, d, J = 16.18 Hz, Ar−CH═C*H*−), 6.28 (1H, d, J = 16.18 Hz, Ar−C*H*═CH−), 7.10 (1H, d, J = 8.62 Hz, Ar−*H*), 7.62 (1H, d, J = 8.62 Hz, Ar−*H*), 8.03 (1H, s, Ar−*H*). ^{13}C NMR (50 MHz): δ 17.72, 23.19, 23.26, 25.76, 41.19, 42.82, 112.56, 120.05, 121.9, 124.60, 130.95, 131.58, 133.57, 135.07, 139.33, 145.23, 154.05. IR: 669, 762, 1019, 1078, 1216, 1322, 1404, 1422, 1537, 1626, 2854, 2924, 3020, 3377 cm^{-1}. MS at m/z 300 ($M^+ - 1$).

*4.1.2.11. Synthesis of 3-amino,4-(3,7-dimethyl-3-vinylocta-1,6-dienyl) phenol (**12**).* A mixture of Fe−Zn (3:3 mmol) and 5% HCl (1 mL) was added to the solution of 3-nitrobakuchiol in DCM (10 mL) with heating (40 °C) and stirring. The reaction mixture was cooled and filtered. The filtrate was extracted with DCM and washed with aqueous saturated $NaHCO_3$ solution. The organic layer was dried and evaporated. The product was purified by column chromatography over silica gel using hexane:EtOAc (17:3) as the eluent to afford **12** in 70% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.20 (3H, s, −C(CH═CH$_2$)C*H*$_3$), 1.47 (2H, m, −C(CH$_3$)C*H*$_2$CH$_2$−), 1.58 and 1.67 (3H each, s, ═C(C*H*$_3$)$_2$), 1.93 (2H, m, −C*H*$_2$CH═), 5.03 (2H, m, −C(CH═C*H*$_2$)CH$_3$, −CH$_2$C*H*═C(CH$_3$)$_2$), 5.09 (1H, t, J = 7.2 Hz, −C*H*═C), 5.87 (1H, dd, J = 17.3, 10.8 Hz,−C(C*H*═CH$_2$)), 5.99 (1H, d, J = 16.27 Hz, Ar−CH═C*H*−), 6.16 (1H, d, J = 16.2 Hz, Ar−C*H*═CH−), 6.62 (1H, d, J = 8.0 Hz, Ar−*H*), 6.65 (1H, d, J = 8.0 Hz, Ar−*H*), 6.79 (1H, s, Ar−*H*). ^{13}C NMR (125 MHz): δ 17.72, 23.19, 23.26, 25.76, 41.19, 42.82, 112.56, 114.4, 115.3, 117.9, 124.8, 126.8, 131.2, 131.4, 134.4, 135.5, 143.6, 146.0. IR: 757, 825, 909, 1020, 1122, 1290, 1382, 1457, 1508, 1637, 2925, 2957 cm^{-1}. MS at m/z 294 ($M^+ + Na$).

*4.1.2.12. Synthesis of 4-(3-ethyl-3,7-dimethyloctyl)-2-iodophenol (**13**).* A mixture of 0.5 mmol of **14** (hexahydrobakuchiol, prepared by hydrogenation of bakuchiol), molecular iodine (0.5 mmol), and CAN (10 mol%) in acetonitrile (5 mL) was stirred at 25 °C and the reaction monitored by TLC. After the completion of the reaction, the contents were treated with aqueous $Na_2S_2O_3$ solution (10 mL), extracted with ethyl acetate (3 × 10 mL), the organic layer washed with water (2 × 10 mL), dried over sodium sulfate and concentrated. The crude product purified by CC over silica gel (60–120 mesh) using hexane:EtOAc (19:1) as the eluent to afford **13** in 88% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.81–0.93 (12H, m), 1.23–1.59 (11H, m), 2.52 (2H, m, Ar–CH_2–), 7.08 (1H, d, J = 8.5 Hz, Ar–*H*), 7.44 (1H, dd, J = 8.5, 2.01 Hz, Ar–*H*), 7.92 (1H, d, J = 2.04 Hz, Ar–*H*). ^{13}C NMR (125 MHz): δ 8.06, 21.25, 22.74, 24.52, 27.98, 29.59, 31.47, 35.29, 39.09, 40.05, 41.10, 119.81, 123.70, 133.38, 136.11, 138.19, 153.33. IR: 668, 764, 824, 1079, 1181, 1247, 1324, 1382, 1427, 1464, 1488, 1539, 158, 1630, 2868, 2931, 2957, 3250 cm^{-1}. MS at m/z 411(M^+ + Na).

*4.1.2.13. Synthesis of 4-(3-ethyl-3,7-dimethyloctyl)phenol (**14**).* The title compound was prepared by hydrogenation of methanolic solution of **1** (1.0 mmol, 25 mL) at 35 psi with Pd/C to afford **14** in 98% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.80–0.89 (12H, m), 1.12–1.61 (11H, m), 2.41 (2H, m, Ph–CH_2–), 6.74 (2H, d, J = 8.3 Hz, 2× Ar–*H*), 7.03 (2H, d, J = 8.3 Hz, 2× Ar–*H*). ^{13}C NMR (100 MHz): δ 8.27, 21.67, 24.48, 27.97, 29.47, 31.66, 35.30, 39.32, 40.20, 41.62, 115.27, 129.32, 136.1, 153.51. IR: 825, 1021, 1170, 1230, 1381, 1462, 1513, 1612, 2867, 2930, 2957, 3356 cm^{-1}. MS at m/z 261 (M^+ − 1).

*4.1.2.14. Synthesis of 6-(4-hydroxystyryl)-2,6-dimethylocta-2,7-dienal (**15**).* Bakuchiol (0.5 g, 1.9 mmol) was dissolved in a mixture of acetic acid and water (9:1), SeO_2 (216 mg, 1.9 mmol) was added and the contents stirred for 4 h at 20 °C. The reaction mixture was diluted with ice-cold water and extracted with EtOAc (2 × 100 mL). The organic layer washed with water (2 × 50 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give crude product, which was purified on silica gel column using Pet. ether 60–80:EtOAc (9:1) as the eluent to afford **15** in 67% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.25 (3H, s, –C(CH=CH_2)CH_3), 1.64 (2H, m, –C(CH_3)CH_2CH_2–), 1.72 (3H, s, =C(CH_3)), 2.01 (2H, m, –CH_2CH=), 4.99 (2H, m, –C(CH=CH_2)CH_3), 5.89 (1H, dd, J = 17.3, 10.8 Hz, –C(C*H*=CH_2)), 6.02 (1H, d, J = 16.28 Hz, Ar–CH=C*H*–), 6.28 (1H, d, J = 16.28 Hz, Ar–C*H*=CH–), 6.50 (1H, t, J = 7.0 Hz, –C*H*=C), 6.78 (2H, d, J = 8.56 Hz, 2× Ar–*H*), 7.24 (2H, d, J = 8.56 Hz, 2× Ar–*H*), 9.36 (1H, s, –C*H*O). ^{13}C NMR (50 MHz): δ 18.5, 24.64, 24.73, 42.65, 43.8, 113.51, 115.56, 127.85, 129.68, 131.81, 137.33, 140.42, 147.35, 152.86, 155.6, 195.1. IR: 757, 1020, 1084, 1120, 1169, 1217, 1456, 1513, 1610, 1715, 2851, 2922, 3384 cm^{-1}. MS at m/z 269 (M^+ − 1).

*4.1.2.15. Synthesis of 12,13-epoxybakuchiol (**16**).* Bakuchiol (5.0 mmol) was dissolved in 30 mL DCM at 0 °C and *m*-CPBA (5.5 mmol) in 60 mL DCM was added slowly. After being stirred for 3 h at 25 °C, aqueous saturated $NaHSO_3$ solution added and the contents stirred for an additional 0.5 h. The reaction mixture was extracted with DCM and the organic layer was washed with water, dried, and evaporated. The product was purified by CC over silica gel using hexane:EtOAc (9:1) as the eluent to afford **16** in 80% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.20 (3H, s, –C(CH=CH_2)CH_3), 1.22 and 1.23 (3H each, s, C(CH_3)$_2$), 1.57 (4H, m, –C(CH_3)CH_2CH_2–), 2.67 (1H, t, J = 6.0 Hz,–CH_2–C*H*–), 5.03 and 5.06 (1H each, m, –C(CH=CH_2)CH_3), 5.93 (1H, m, –C*H*=CH_2), 6.10 (1H, d, J = 16.2 Hz, Ar–CH=C*H*–), 6.31 (1H, d, J = 16.2 Hz, Ar–C*H*=CH–), 6.79 (2H, d, J = 8.5 Hz, 2× Ar–*H*), 7.31 (2H, d, J = 8.5 Hz, 2× Ar–*H*). ^{13}C NMR (50 MHz): δ 18.3, 23.1, 23.2, 24.6, 37.9, 42.3, 57.8, 64.2, 112.0, 115.7, 127.7, 127.8, 129.8, 134.7, 146.4, 157.4. IR: 814, 912, 970, 1037, 1174, 1248, 1462, 1510, 1608, 1632, 2854, 2915, 2965, 3384. MS at m/z 271 (M^+ − 1).

*4.1.2.16. Synthesis of 4-[2-{4-(2-hydroxypropan-2-yl)-1,2-dimethylcyclopentyl}vinyl]phenol (**17**).* Bakuchiol (1.95 mmol) dissolved in THF:H_2O (1:1) (10 mL) at 0 °C, added $Hg(OAc)_2$ (0.625 g, 1.95 mmol), after stirring the solution for 10 min, 10% NaOH solution was added to the reaction mixture. Reaction mixture was further stirred for half an hour, followed by addition of $NaBH_4$, reaction monitored through TLC. After the completion of the reaction, the contents neutralized with dilute HCl and extracted with EtOAc (3 × 100 mL). The combined organic layers washed with water (2 × 50 mL), and dried over anhydrous Na_2SO_4. The crude product purified by CC over silica gel using Hexane:EtOAc (9:1) as the eluent to afford **17** in 90% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.95 (3H, d, J = 6.67 Hz, –CHCH_3), 0.98 (3H, s, –C–CH_3), 1.24 (6H, br s, –C–(CH_3)$_2$), 1.49 (1H, q, J = 8.6 Hz, 2.2 Hz, –C*H*), 1.59 (2H, m, –CH_2), 1.72 (2H, m, CH–CH_2–CH_2) 1.87 (1H, m,–CH–(CH_3)$_2$OH), 6.03 (1H, d, J = 16.2 Hz, Ar–CH=C*H*–), 6.24 (1H, d, J = 16.2 Hz, Ar–C*H*=CH–), 6.76 (2H, d, J = 8.5 Hz, Ar–*H*), 7.22 (2H, d, J = 8.5 Hz, Ar–*H*). ^{13}C NMR (125 MHz): δ 16.07, 18.48, 26.12, 27.68, 28.40, 39.80, 44.51, 48.20, 55.62, 74.27, 115.47, 125.81, 127.23, 130.58, 137.97, 154.98. IR: 757, 816, 854, 928, 968, 1021, 1164, 1237, 1379, 1451, 1512, 1608, 2927, 2963, 3360 cm^{-1}. MS at m/z 273 (M^+ − 1).

*4.1.3. General procedure for preparation of compounds **18**–**22** by Heck coupling reaction*

A mixture of substituted aromatic halide (1.2 equiv), bakuchiol (1.0 equiv) and K_2CO_3 (2 equiv) in dry DMF in presence of tetrakis (triphenylphosphine) palladium (O) complex was refluxed under N_2 current at 140 °C for 7 h. The contents cooled, diluted with 5% HCl to attain pH 5, and extracted with diethyl ether (3 × 100 mL), the organic layer washed with water (2 × 50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude product purified by CC over silica (60–120) with hexane:EtOAc mixture as the eluent to afford the required compound.

*4.1.3.1. Synthesis of 4-{3-(2-methoxystyryl)-3,7-dimethylocta-1,6-dienyl}phenol (**18**).* Using 2-iodoanisole as the substrate, the title compound was prepared by the procedure as described under Section 4.1.3 and the crude product purified by CC over silica gel using hexane:EtOAc (17:3) as the eluent to afford **18** in 52% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.33 (3H, s, –C–CH_3), 1.63 (2H, m, –C(CH_3)CH_2CH_2), 1.65 and 1.69 (3H each, s, =(CH_3)$_2$), 2.03 (2H, m, –CH_2CH=), 3.84 (3H, s, Ar–OCH_3), 5.13 (1H, t, J = 6.9 Hz,–C*H*=C), 6.25 (2H, m, Ar–CH=C*H*–, Ar′–CH=C*H*–), 6.81 (5H, m, 2× Ar–C*H*=CH, 2× Ar–*H*, Ar–*H*), 7.23 (4H, m, 2× Ar–*H*, 2× Ar′–*H*), 7.46 (1H, d, J = 8.4 Hz, Ar′–*H*). ^{13}C NMR (50 MHz): δ 18.11, 23.83, 24.45, 26.13, 42.16, 44.22, 55.91, 111.33, 115.78, 121.06, 122.15, 125.37, 126.75, 126.94, 127.84, 127.85, 128.40, 132.1, 132.4, 136.7, 138.9, 156.1, 158.7. IR: 757, 925, 1040, 1064, 1233, 1371, 1457, 1509, 1578, 1606, 2853, 2924, 2959, 3411 cm^{-1}. MS at m/z 361 (M^+ − 1).

*4.1.3.2. Synthesis of 4-{3-(4-methoxystyryl)-3,7-dimethylocta-1,6-dienyl}phenol (**19**).* The title compound was prepared using the procedure as described under Section 4.1.3 by coupling Bakuchiol **1** with 4-iodoanisole and the product purified by CC over silica gel using hexane:EtOAc (17:3) as the eluent to afford **19** in 55% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.28 (3H, s, –C–CH_3), 1.57 (2H, m, =CHCH_2CH_2), 1.59 and 1.67 (3H each, s, =C(CH_3)$_2$), 1.98 (2H, m, =CHCH_2–), 3.79 (3H, s, Ar–OCH_3), 5.12 (1H, t, J = 6.9 Hz, –CH_2C*H*), 6.11 (2H, d, J = 16.2 Hz, Ar–CH=C*H*–, Ar′–CH=C*H*–), 6.30 (2H, d, J = 16.2 Hz, Ar–C*H*=CH–, Ar′–C*H*=CH–), 6.76 (2H, d, J = 8.5 Hz, 2× Ar–*H*), 6.85 (2H, d, J = 8.7 Hz, 2× Ar′–*H*), 7.26 (2H, d, J = 6.85 Hz,

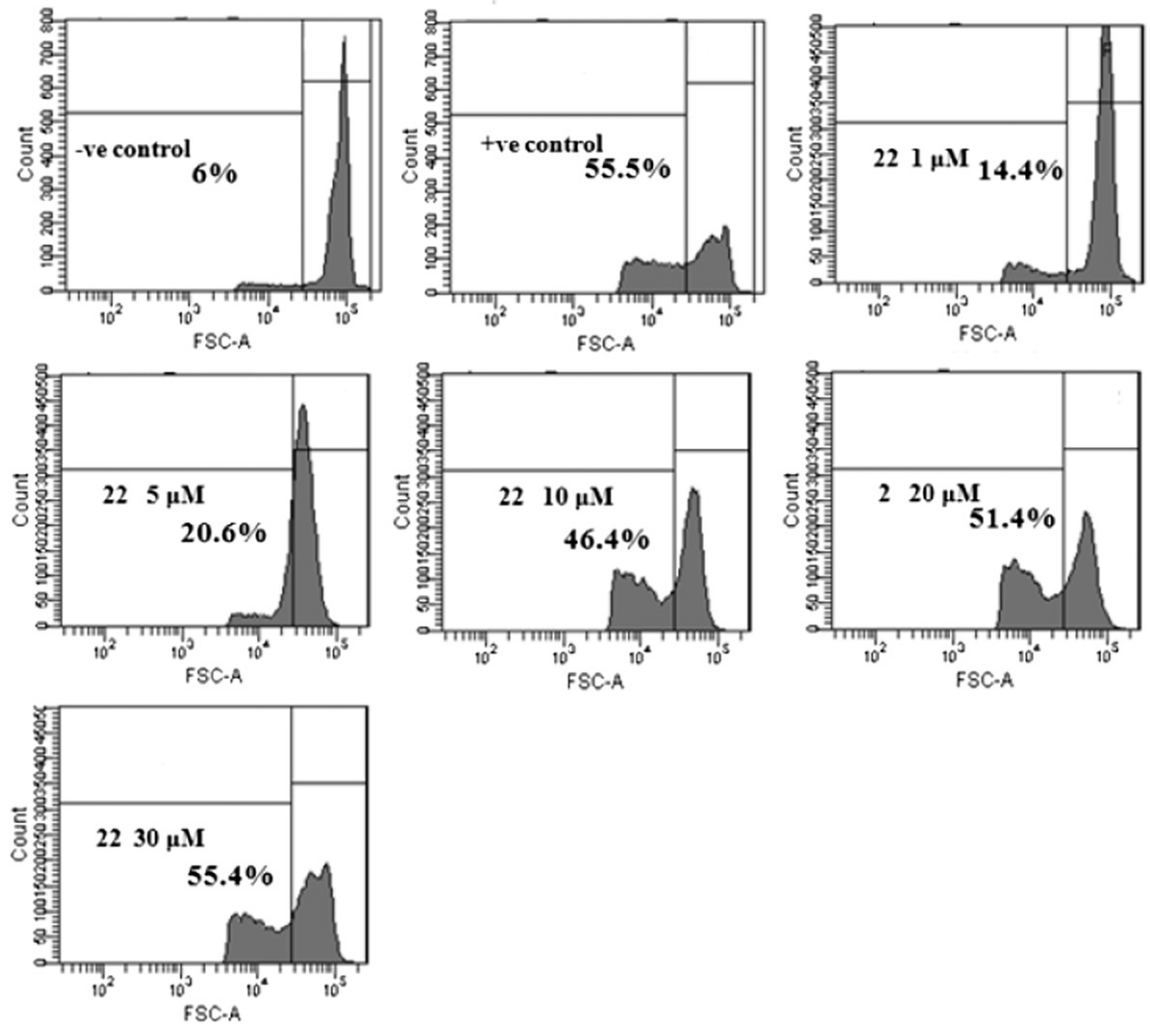

Fig. 9. Compound **22** induced loss of mitochondrial membrane potential ($\Delta\Psi_m$) HL-60. Cells (1×10^6/mL/ well) incubated with **22** at different conc. in 6 well plate for 24 h. Before 1 h of the completion of the experiment cells were treated with Rodamine-123 (5 μM). Cells were washed with PBS and centrifuged and finally dissolved in 1 mL of PBS and analyzed on flow cytometer. Data are representative of one of two similar experiments.

2× Ar–*H*), 7.32 (2H, d, $J = 8.7$ Hz, 2× Ar′–*H*). ^{13}C NMR (50 MHz): δ 18.10, 23.81, 24.50, 26.13, 42.22, 42.49, 55.75, 114.40, 115.83, 125.31, 127.00, 127.65, 127.84, 131.18, 131.19, 131.21, 136.54, 155.1, 159.5. IR: 817, 970, 1035, 1098, 1246, 1439, 1511, 1608, 2854, 2925, 2963, 3030, 3159 cm^{-1}. MS at *m*/*z* 361 ($M^+ - 1$).

*4.1.3.3. Synthesis of 4-{3-(3-formyl,4-methoxystyryl)-3,7-dimethylocta-1,6-dienyl}phenol (**20**).* The title compound prepared by coupling Bakuchiol with 2-formyl, 4-iodoanisole and purified by the procedure as described under Section 4.1.3.1 to afford **20** in 63% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.29 (3H, s, –C–CH_3), 1.57 (2H, m, =$CHCH_2CH_2$–), 1.59 and 1.67 (3H each, s, $C(CH_3)_2$), 1.97 (2H, m, =CCH_2), 3.92 (3H, s, OCH_3), 5.12 (1H, t, $J = 6.9$ Hz, –*CH*=C), 6.21 (4H, m, 2× Ar–CH=*CH*–, 2× Ar′–CH=*CH*–), 6.78 (2H, d, $J = 8.6$ Hz, 2× Ar–*H*), 6.93 (1H, d, $J = 8.6$ Hz, Ar′–*H*),7.26 (4H, d, $J = 8.6$ Hz, 2× Ar–*H*), 7.54 (1H, d, $J = 8.6$ Hz, Ar′–*H*), 7.85 (1H, s, Ar′–*H*), 10.45 (1H, s, CHO). ^{13}C NMR (125 MHz): δ 17.71, 23.39, 23.93, 25.72, 41.72, 42.19, 55.92, 111.86, 115.5, 124.61, 125.48, 126.88, 127.43, 130.89, 131.43, 132.17, 135.45, 138.0, 155.13, 160.99, 190.32. IR: 757, 816, 970, 1015, 1101, 1171, 1234, 1375, 1446, 1511, 1609, 2854, 2925, 2961, 3022, 3363 cm^{-1}. MS at *m*/*z* 389 ($M^+ - 1$).

*4.1.3.4. Synthesis of 4-{3-(4-hydroxystyryl)-3,7-dimethylocta-1,6-dienyl} phenol (**21**).* This compound was prepared and purified by the same procedure as described under Section 4.1.3.1 to afford **21** in 64% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.28 (3H, s, –C–CH_3), 1.57 (2H, m, =$CHCH_2CH_2$–), 1.59 and 1.67 (3H each, s, $C(CH_3)_2$), 2.0 (2H, m, =CCH_2CH_2), 5.14 (1H, t, $J = 6.9$ Hz, –*CH*=C), 6.11 (2H, d, $J = 16.2$ Hz, 2× Ar–CH=*CH*–), 6.29 (2H, d, $J = 16.2$ Hz, 2× Ar–*CH*=*CH*–), 6.77 (4H, d, $J = 8.6$ Hz, 4× Ar–*H*), 7.26 (4H, d, $J = 8.6$ Hz, 2× Ar–*H*). ^{13}C NMR (125 MHz): δ 17.71, 23.39, 24.03, 25.74, 41.77, 42.06, 115.45, 124.87, 126.54, 127.43, 130.81, 131.40, 136.08, 154.7. IR: 757, 816, 970, 1015, 1101, 1171, 1234, 1375, 1446, 1511, 1609, 2854, 2925, 2961, 3022, 3363 cm^{-1}. MS at *m*/*z* 347 ($M^+ - 1$).

*4.1.3.5. Synthesis of 4-{3-(4-methoxystyryl)-3,7-dimethylocta-1,6-dienyl}phenol (**22**).* This compound was prepared (by coupling methyl ether Bakuchiol with 4-iodophenol) and purified by the procedure as described under Section 4.1.3.1 to afford **22** in 58% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.32 (3H, s, –C–CH_3), 1.62 (2H, m, –CH_2CH_2CH=), 1.64 and 1.71 (3H each, s, =$C(CH_3)_2$), 2.04 (2H, m, –CH_2CH=), 3.83 (3H, s, Ar–OCH_3), 5.17 (1H, t, $J = 6.9$ Hz,–CH_2CH=), 6.17 (2H, d, $J = 16.2$ Hz, 2× Ar′–CH=*CH*–),

6.33 (2H, d, $J = 16.2$ Hz, 2× Ar−C*H*═CH), 6.80 (2H, d, $J = 8.6$ Hz, 2× Ar−*H*), 6.88 (2H, d, $J = 8.6$ Hz, 2× Ar′−*H*), 7.29 (2H, d, $J = 8.6$ Hz, 2× Ar−*H*), 7.34 (2H, d, $J = 8.6$ Hz, 2× Ar′−*H*). ^{13}C NMR (125 MHz): δ 16.73, 22.42, 23.10, 24.74, 40.83, 41.09, 54.37, 113.01, 114.46, 123.92, 125.61, 126.26, 126.44, 129.79, 129.81, 130.35, 135.08, 135.18, 153.85, 157.73. IR: 817, 970, 1034, 1102, 1173, 1246, 1302, 1374, 1441, 1511, 1608, 2853, 2925, 2964, 3030, 3305 cm^{-1}. MS at *m*/*z* 361 ($M^+ - 1$).

*4.1.4. General procedure for preparation of compounds **23**–**25**, **27** by oxidative Heck coupling reaction*

Bakuchiol dissolved in DMF (0.2 M) stirred at room temperature (<25 °C) and to the clear solution, substituted phenyl boronic acid (1.2 equiv) followed by a single addition of Na_2CO_3 and $Pd(OAc)_2$ catalyst. The reaction flask fitted with an oxygen balloon, heated up to 100 °C with stirring for 7 h. The contents diluted with diethyl ether (20 mL), and washed with aqueous NaCl solution (2 × 25 mL). The organic layer dried over Na_2SO_4 and filtered. The filtrate concentrated under reduced pressure and the crude products purified by CC over silica gel (60−120 mesh) with EtOAc:Hexane mixture as the eluent to afford the appropriate compounds.

*4.1.4.1. Synthesis of 4-{3-(4-fluorostyryl)-3,7-dimethylocta-1,6-dienyl}phenol (**23**).* The title compound prepared by the method as described above by coupling of 4-fluorophenyl boronic acid and compound **1** and purified by CC over silica gel using hexane:EtOAc (9:1) as the eluent to afford **23** in 51% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30 (3H, s, −C−C*H*$_3$), 1.58 (2H, m, ═CC*H*$_2$CH$_2$−), 1.59 and 1.68 (3H each, s, ═C(C*H*$_3$)$_2$), 2.00 (2H, m,−CH$_2$C*H*$_2$CH═), 5.13 (1H, t, $J = 6.9$ Hz, −C*H*═C), 6.13 and 6.18 (1H each, d, $J = 16.2$ Hz, 2× Ar−CH═C*H*−), 6.30 and 6.33 (1H each, d, $J = 16.2$ Hz, 2× Ar−C*H*═CH), 6.78 (2H, d, $J = 8.4$ Hz, 2× Ar−*H*), 6.99 (2H, d, $J = 8.6$ Hz, 2× Ar−*H*), 7.26 (2H, d, $J = 8.4$ Hz, 2x Ar−*H*), 7.33 (2H, d, $J = 8.6$ HZ, 2× Ar′−*H*). ^{13}C NMR (125 MHz): δ 18.40, 24.07, 24.65, 26.42, 42.41, 42.85, 115.74, 116.12, 125.45, 126.79, 127.46, 128.13, 128.20, 131.41, 132.16, 134.69, 136.39, 138.68, 155.51, 163.66. IR: 757, 1018, 1123, 1157, 1216, 1403, 1456, 1508, 1602, 2850, 2920, 2960, 3405 cm^{-1}. MS at *m*/*z* 349 ($M^+ - 1$).

*4.1.4.2. Synthesis of 4-{3-(4-aminostyryl)-3,7-dimethylocta-1,6-dienyl}phenol (**24**).* The title compound prepared by the method as described under Section 4.1.4.1 by coupling 4-iodobenzamine and compound **1** and purified by CC over silica gel using hexane:EtOAc (9:1) as the eluent to afford **24** in 51.7% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.25 (3H, s, −C−C*H*$_3$), 1.57 (2H, m, −C*H*$_2$CH$_2$CH═), 1.58 and 1.67 (3H each, s, ═C(C*H*$_3$)$_2$), 1.99 (2H, m, ═CHC*H*$_2$), 5.13 (1H, t, $J = 6.9$ Hz, −C*H*═C), 6.07 and 6.10 (1H each, d, $J = 16.2$ Hz, 2× Ar−CH═C*H*), 6.25 and 6.27 (1H, d, $J = 16.2$ Hz, 2× Ar−C*H*═CH), 6.64 (2H, d, $J = 8.6$ Hz, 2× Ar−*H*), 6.75 (2H, d, $J = 8.4$ Hz, 2× Ar′−*H*), 7.19 (2H, d, $J = 8.4$ Hz, 2× Ar−*H*) 7. 24 (2H, d, $J = 8.6$ Hz, 2× Ar′−*H*). ^{13}C NMR (125 MHz): δ 17.68, 23.40, 24.15, 25.70, 41.85, 41.99, 115.48, 124.78, 124.96, 126.45, 126.53, 126.91, 127.20, 127.38, 129.75, 131.88, 132.65, 134.76, 136.27, 146.1, 154.85. IR: 617, 655, 911, 972, 1014, 1110, 1195, 1369, 1412, 1452, 1505, 1603, 1633, 2855, 2922, 2967, 3033 cm^{-1}. MS at *m*/*z* 346 ($M^+ - 1$).

*4.1.4.3. Synthesis of 4-{-3-(3-chloro-4-propoxystyryl)-3,7-dimethylocta-1,6-dienyl}phenol (**25**).* The title compound prepared and purified by the method as described under Section 4.1.4.1 by coupling of 3-chloro-4-propoxylphenyl boronic acid and compound **1** and purified by CC over silica gel using hexane:EtOAc (9:1) as the eluent to afford **25** in 49.6% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.06 (3H, t, $J = 7.3$ Hz, −CH$_2$C*H*$_3$), 1.28 (3H, s, −C−C*H*$_3$), 1.57 (2H, m, C*H*$_2$CH$_2$CH═), 1.59 and 1.67 (3H each, s, ═C(C*H*$_3$)$_2$), 1.92 (4H, m, −C*H*$_2$CH$_3$, −C*H*$_2$CH═), 3.95 (2H, t, $J = 6.5$ Hz,−OC*H*$_2$−), 5.07 (1H, t, $J = 6.9$ Hz, −CH$_2$C*H*═C), 6.15 (4H, m, 2× Ar−C*H*═CH−, 2× Ar′−CH═C*H*−), 6.80 (3H, m, 1× Ar′−*H*, 2× Ar−*H*), 7.21 (3H, m, 2× Ar−*H*, 1× Ar′−*H*), 7.41 (1H, s, 1× Ar′−*H*). ^{13}C NMR (125 MHz): δ 14.24, 18.7, 24.09, 24.15, 25.75, 27.8, 41.80, 42.12, 71.4, 114.06, 115.50, 122.50, 124.12, 126.10, 126.46, 126.74, 127.38, 127.41, 128.10, 130.81, 131.78, 132.2, 135.78, 136.66, 152.20, 155.32. IR: 762, 803, 909, 1108, 1258, 1402, 1462, 1500, 1602, 2852, 2922, 3385 cm^{-1}. MS at *m*/*z* 423 ($M^+ - 1$).

*4.1.4.4. Synthesis of 4-{3-(3-(hydroxymethyl)-4-methoxystyryl)-3,7-dimethylocta-1,6-dienyl}phenol (**26**).* Sodium borohydride (0.5 mmol) reduction of compound **20** (1 mmol) in methanol at 0 °C afforded title compound on purification by CC over silica gel using hexane:EtOAc (9:1) as the eluent in 97% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.29 (3H, s, −C−C*H*$_3$), 1.56 (2H, m, −C*H*$_2$CH$_2$CH═), 1.59 and 1.67 (3H each, s, ═C(C*H*$_3$)$_2$), 1.99 (2H, m, −CH$_2$C*H*$_2$CH═), 3.82 (3H, s, Ar−OC*H*$_3$), 4.69 (2H, s, ArC*H*$_2$OH), 5.11 (1H, t, $J = 6.9$ Hz,−CH$_2$C*H*═C), 6.12 (2H, d, $J = 16.2$ Hz, 2× Ar−CH═C*H*), 6.28 (2H, d, $J = 16.2$ Hz, 2× Ar−C*H*═CH), 6.75 (2H, d, $J = 8.4$ Hz, 2× Ar−*H*), 6.81 (1H, d, $J = 8.6$ Hz, Ar′−*H*), 7.24 (3H, m, 2× Ar−*H*, Ar′−*H*), 7.32 (1H, s, Ar′−*H*). ^{13}C NMR (125 MHz): δ 17.74, 23.43, 24.09, 25.75, 41.80, 42.12, 55.49, 62.26, 110.40, 115.50, 124.92, 126.42, 126.46, 126.74, 126.97, 127.38, 127.41, 128.7, 130.81, 131.35, 135.78, 136.66, 155.20, 156.67. IR: 757, 813, 970, 1033, 1107, 1251, 1375, 1461, 1503, 1512, 1609, 2853, 2961, 3332 cm^{-1}. MS at *m*/*z* 391 ($M^+ - 1$).

*4.1.4.5. Synthesis of 1-methoxy-[4-{3-(4-methoxystyryl)-3,7-dimethylocta-1,6-dienyl}] benzene (**27**).* The title compound prepared by coupling of compound **10** with 4-iodoanisole following the procedure as described under Section 4.1.4.1 and purified by CC over silica gel using hexane:EtOAc (9:1) as the eluent to afford **27** in 66% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (3H, s, −C−C*H*$_3$), 1.52 (2H, m, −C*H*$_2$CH$_2$CH═), 1.54 and 1.67 (3H each, s, ═C(C*H*$_3$)$_2$), 1.97 (2H, m, −C*H*$_2$CH═), 3.83 (6H, s, 2× Ar−OC*H*$_3$), 5.11 (1H, t, $J = 6.9$ Hz, −CH$_2$C*H*═C), 6.13 (2H, d, $J = 16.2$ Hz, Ar−CH−C*H* and Ar′−CH−C*H*), 6.30 (2H, d, $J = 16.2$ Hz, Ar−C*H*═CH and Ar′−C*H*═CH), 6.85 (4H, d, $J = 8.34$ Hz, 2× Ar−*H* and 2× Ar′−*H*), 7.30 (4H, d, $J = 8.34$ Hz, 2× Ar−*H* and 2× Ar′−*H*). ^{13}C NMR (100 MHz): δ 17.68, 23.39, 24.10, 25.71, 41.79, 42.07, 55.21, 113.95, 124.89, 126.64, 127.24, 130.73, 131.30, 136.11, 158.82. IR: 756, 814, 970, 1014, 1100, 1173, 1234, 1376, 1444, 1511, 1609, 2852, 2924 cm^{-1}. MS at *m*/*z* 375 ($M^+ - 1$).

*4.1.4.6. Synthesis of 2,6-dibromo-4-(3-ethyl-3,7-dimethyloctyl)phenol (**28**).* Bromine (0.5 mmol) was added to CCl_4 solution (10 mL) of **14** (hexahydro bakuchiol) (0.5 mmol), the contents stirred for 2 h, and the reaction monitored by TLC. The reaction mixture on usual work up and purification by CC over silica gel using hexane:EtOAc (19:1) as the eluent to afford **28** in 96% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.80 (3H, t, $J = 7.5$ Hz, C*H*$_3$−CH$_2$), 0.83 (6H, d, $J = 6.5$ Hz, (C*H*$_3$)$_2$−CH), 0.85 (3H, s, C*H*$_3$−C), 1.05−1.45 (11H, m), 2.34−2.43 (2H, m, Ph−C*H*$_2$−), 7.24 (2H, br s, 2× Ar−*H*). ^{13}C NMR (125 MHz): δ 8.04, 21.21, 22.73, 24.46, 27.94, 29.05, 31.43, 35.23, 39.07, 40.03, 41.22, 109.56, 131.70, 138.39, 147.22. IR: 735, 862, 1019, 1197, 1239, 1271, 1319, 1382, 1407, 1472, 1652, 2867, 2930, 2956, 3508 cm^{-1}. MS at *m*/*z* 413 ($M^+ - 1$).

4.2. Biology

RPMI-1640 medium (#N 3520), rhodamine-123 (Rh-123) (#R 8004), propidium iodide (PI) (#P4170), proteinase-K, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (#M 2128), penicillin (#P 3032), streptomycin (#S 9137), camptothecin (#C 911), fetal bovine serum (# 7524), mitomycin (#M 4287), sodium bicarbonate (#S 5761), phosphate buffer saline (PBS) (#P 3831), sulphorhodamine (SRB) (#1402), trypsin (#T 4799), paclitaxel

(#T 7402), 5 fluorouracil (5-FU) (#F 6627), doxorubicin (#D 1515), gentamycin sulfate (#G 1264) were procured from Sigma–Aldrich. Tris buffer (#RM 262), electrophoresis reagents, bromophenol blue (#RM 117) were procured from Himedia while Glacial acetic acid (#21055) was purchased from Fisher Scientific. Trichloroacetic acid was purchased from Merck Specialties Private Ltd.

4.2.1. Cell culture, growth conditions and treatment

Human promyelocytic leukemia cell line (HL-60), human ovarian cancer cell line (OVCAR-5), human neuroblastoma cancer cell line (IMR-32), were procured from National Centre for Cell Sciences (NCCS), Pune, India. Human lung carcinoma cell line (A-549) was obtained from National Cancer Institute, Frederick, USA. Human prostate cancer cell line (PC-3) was obtained from National Cancer Institute (NCI), Bethesda, USA. Cells were grown in RPMI-1640/ MEM medium containing 10% FCS, 100 unit penicillin/ 100 μg streptomycin per mL medium. Cells were grown in CO_2 incubator (Thermo Scientific, USA) at 37 °C with 98% humidity and 5% CO_2 gas environment. Cells were treated with different structural analogs of bakuchiol dissolved in DMSO while the untreated control cultures received only the vehicle (DMSO, <0.2%).

4.2.2. Sulpharhodamine B assay for % growth inhibition

The Sulpharhodamine B (SRB) assay was used to evaluate inhibitory effect of different structural analogs of bakuchiol. The assay relies on the ability of SRB to bind to protein components of cells that have been fixed to tissue-culture plates by trichloroacetic acid (TCA). SRB is a bright-pink aminoxanthene dye with two sulfonic groups that bind to basic amino-acid residues under mild acidic conditions, and dissociate under basic conditions. As the binding of SRB is stoichiometric, the amount of dye extracted from stained cells is directly proportional to the cell mass. To determine the effect of different structural analogs of bakuchiol on cell number over time, SRB assays were performed as described. Cells were seeded in flat-bottomed 96-well plates. The cells were allowed to adhere overnight, and then media containing samples were added at different concentrations. The plates were incubated for 48 h. The cells were fixed by adding 50 μL per well of ice-cold 50% TCA to each well for 60 min. The plates were washed five times in running tap water and stained with 100 μL per well SRB reagent (0.4% w/v SRB in 1% acetic acid for 30 min). The plates were washed five times in 1% acetic acid to remove unbound SRB and allowed to dry overnight. SRB was solubilized with 100 μL per well 96-well plate 10 mM Tris-base, shaken for 5 min and the OD was measured at 570 nm with reference wavelength of 620 nm [17]. Further the IC_{50} values on the cancer cells of different tissue origin used for screening were determined by non-linear regression analysis using Graph Pad Software (2236 Avenida de la Playa La Jolla, CA 92037, USA).

4.2.3. Cell proliferation assay

1.5×10^4 HL-60cells/well/100 μL in the logarithmic phase of growth were grown in 96-well plates and exposed to indicate concentrations of compounds **17** and **22** for 48 h. Thereafter, 20 μL of MTT solution (2.5 mg/mL) was added to each well and incubated at 37 °C for 3–4 h in a humidified atmosphere containing 5% CO_2. The plates were then centrifuged at 1500 rpm for 15 min and the supernatant was discarded while the MTT–formazan crystals were dissolved in 150 μL of DMSO. The OD measured at 570 nm with reference wavelength of 620 nm [18].

4.2.4. DNA agarose gel electrophoresis for evaluating DNA fragmentation

HL-60 cells (2×10^6 cells/well/2 mL) were grown in 6 well plates and treated with an indicated concentration of compounds **17** and **22** for 24 h. After treatments cells were centrifuged at 1500 rpm for 10 min, and washed in PBS. The pellet was lysed in 250 μL of lysis buffer (100 mM NaCl, 5 mM EDTA, 10 mM Tris–HCl, pH 8.0, 5% Triton X-100) containing (200 μg/mL) proteinase-K and incubated at 50 °C for 1 h followed by 90 min incubation with 400 μg/mL DNase-free RNase. The DNA was extracted with 100 μL phenol:-chloroform:isoamylalcohol (25:24:1) and centrifuged. DNA was precipitated from aqueous phase with 3 volumes of chilled alcohol and 0.3 M sodium acetate at 20 °C overnight. The precipitate was centrifuged at 13,000 × *g* for 10 min. The DNA pellet was washed in 80% alcohol, dried, dissolved in 50 μL TE buffer, mixed in loading buffer and electrophoresed in 1% agarose gel at 80 V for 1.5 h in TAE buffer [19].

4.2.5. DNA content and cell cycle phase distribution

HL-60 cells (2×10^6cells/well/2 mL) were seeded in 6 well plates and treated for 24 h with 1 μM, 5 μM 10 μM, 20 μM and 30 μM conc. of compound **17** and compound **22**. After 24 h cells were collected, washed in PBS and fixed in 70% cold ethanol for 30 min. Cells were again washed with PBS, subjected to RNase digestion (400 μg/mL) at 37 °C for 45 min. Finally, cells were incubated with propidium iodide (10 μg/mL) [18]. Cells are then analyzed immediately on flow cytometer FACS Aria (Becton Dickinson, USA). The fluorescence intensity of sub-G0 cell fraction represents the apoptotic cell population [20].

4.2.6. Measurement of mitochondrial membrane potential for cellular energy status

Changes in mitochondrial transmembrane potential ($\Delta\Psi_m$) as a result of mitochondrial perturbation were measured after staining with Rhodamine-123 [18,21]. HL-60 cells (1×10^6/mL/well) were grown in 6 well plates and treated with indicated concentration of compounds **17** and **22**. Rh-123 (5 μM) was added 1 h before the termination of experiment. Cells were washed in PBS and centrifuged at 1500 rpm for 5 min, suspended in PBS and analyzed for loss in mitochondrial membrane potential on flow cytometer.

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