European Journal of Medicinal Chemistry 63 (2013) 782–792

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis of 3-*O*-propargylated betulinic acid and its 1,2,3-triazoles as potential apoptotic agents

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ARTICLE INFO

Article history:
Received 1 December 2012
Received in revised form
11 March 2013
Accepted 16 March 2013
Available online 26 March 2013

Keywords:
Betulinic acid 1,2,3-trizole derivatives
Click chemistry
Cytotoxicity
DNA fragmentation
Apoptosis

ABSTRACT

Cytotoxic agents from nature are presently the mainstay of anticancer chemotherapy, and the need to reinforce the arsenal of anticancer agents is highly desired. Chemical transformation studies carried out on betulinic acid, through concise 1,2,3-triazole synthesis via click chemistry approach at C-3position in ring A have been evaluated for their cytotoxic potentiation against nine human cancer cell lines. Most of the derivatives have shown higher cytotoxic profiles than the parent molecule. Two compounds i.e. 3{1*N*(2-cyanophenyl)-1*H*-1,2,3-triazol-4yl}methyloxy betulinic acid (**7**) and 3{1*N*(5-hydroxy-naphth-1yl)-1*H*-1,2,3-triazol-4yl}methyloxy betulinic acid (**13**) displayed impressive IC_{50} values (2.5 and 3.5 μM respectively) against leukemia cell line HL-60 (5–7-fold higher potency than betulinic acid). As evident from various biological end points, inhibition of cell migration and colony formation, mitochondrial membrane disruption followed by DNA fragmentation and apoptosis, is demonstrated.

1. Introduction

Natural products (NPs) and the molecules based on NP scaffolds being the proven source of therapeutic agents have increasingly attracted the interests of researchers involved in the area of cancer therapy. In fact, the majority of anticancer and anti-infectious agents are of natural origin [1]. Pentacyclic triterpenoid class of compounds is found pharmacologically active scaffold, and provides privileged motifs for further modifications and structure activity relationship (SAR) analyses [2,3]. To date several reports are available wherein simple or advanced modifications have been performed for the desired biological properties [4–7]. Betulinic acid, a naturally occurring lupane triterpenoid is reported to exhibit anti-cancer, antibacterial and anti-HIV activities [7,8]. The induction of cytotoxicity involves modulation of Bcl-2 family, cell cycle regulatory proteins, regulation of the nuclear factor kappa B (NFκB), as well as inhibition of aminopeptidase N, and growth factor-induced angiogenesis [9,10]. The NP has been extensively studied in xenograft mouse models for its cytotoxic effect against primary cancer cells isolated tumor specimens obtained from glioblastoma and leukemia [10] and the molecule is currently undergoing clinical study at national cancer institute (NCI) [11].

In continuation of our interest involving structural modification of natural products [12,13] for better efficacy, lesser toxicity, betulinic acid was chosen for chemical modification targeting position-3 of ring A, one of the hot spots of the molecule (Fig. 1) in a bid to arrive at a more potent analog having some possible clinical utility and application. Triazole compounds in biological system show binding ability with a variety of enzymes and receptors via diverse non-covalent interactions and display versatile biological activities and many of them have been identified as clinical drugs or candidates for the treatment of various types of diseases [14–16]. These have shown their large development value and wide potential as therapeutic agents such as anti-infective, anticancer, antiviral, and anti-hypertensive agents. In the present paper, the preparation of C_3-aryl substituted 1,2,3-triazoles of betulinic acid and their cytotoxic profiles is described.

2. Result and discussion

Betulinic acid (**1**) is reported as cytotoxic against several human cancer cell lines with $IC_{50} > 40$ baring one or two cell lines where

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http://dx.doi.org/10.1016/j.ejmech.2013.03.028

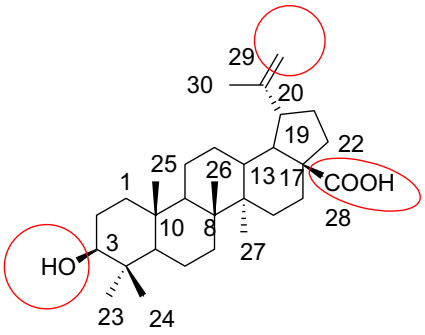
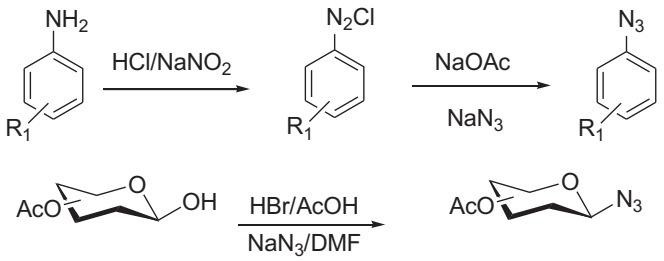


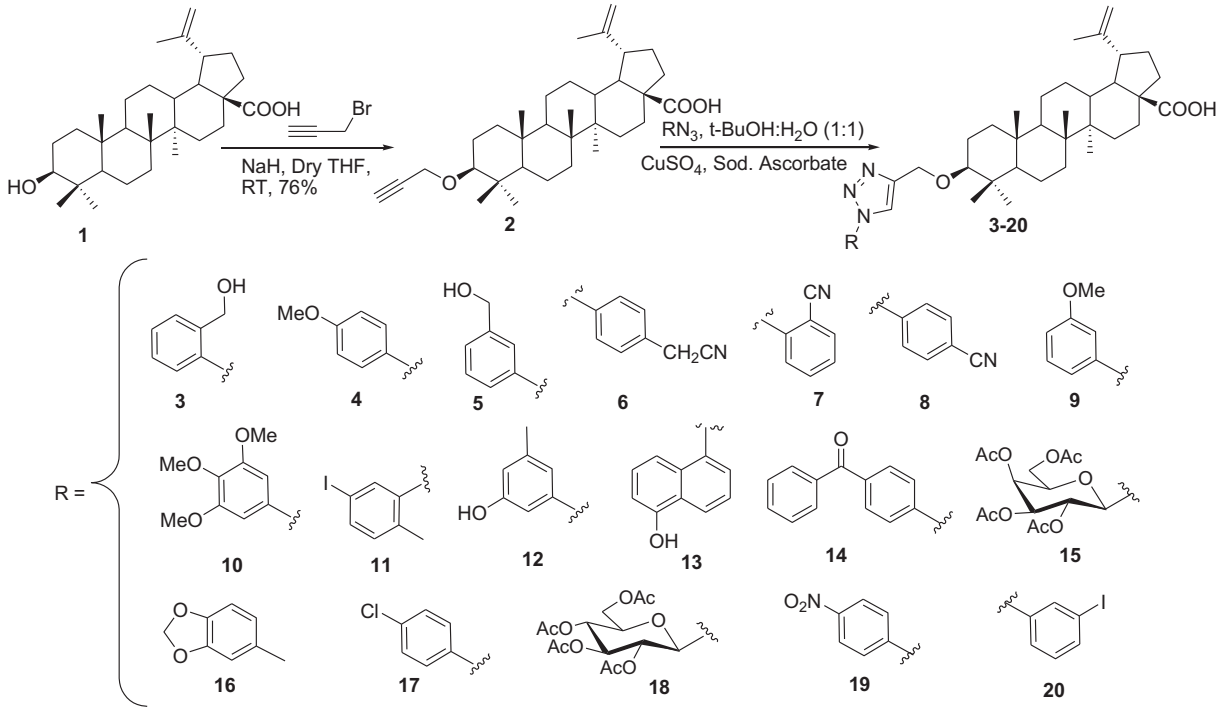
Fig. 1. Chemical structure of betulinic acid.



Scheme 2. Synthesis of substituted aryl azides and acetylated sugar azides.

single digit IC_{50} value is observed [8,10]. The molecule offers several hot spots (e.g. C-3, C-20 and C-28) to provide enough space for chemical modification (Fig. 1). We selected C-3 site as this center is shown to play a lesser role in anti-cancer activity compared to other two important sites [8]. One of the goals of cancer chemotherapy is to explore and develop discovery leads that can selectively induce apoptosis in cancer cells [17]. The strategy envisaged in the present study involved the prologue of nitrogen atom to generate molecules bearing heterocyclic moiety. To achieve this, preparation of 1,2,3-triazole derivatives was undertaken and click chemistry strategy was adopted on the 3-propargyloxy betulinic acid (**2**) (prepared as per Scheme 1) and made to react with appropriate azide (prepared as per Scheme 2). A library of 18 triazoles derivatives bearing structural diversity were thus prepared (Scheme 1) and their chemical identity established by spectral analysis. The overall chemical yields of the synthesized compounds ranged from 92 to 98%.

Compound **1** and its derivatives (**3**–**20**) were screened against a panel of nine human cancer cell lines namely A-549, PC-3, DU-145, SF-295, MCF-7, THP-1, HCT-15 and HL-60 cells (Table S1, Fig. S1) to assess their cytotoxic potential using sulforhodamine B (SRB) assay. In the primary screening of these compounds against cancer cell lines at 50 μM concentration for 48 h, most of the cell lines were found to be sensitive to betulinic acid and its derivatives with many of them exhibiting ≥90 inhibition against various cancer cell lines (Table S1). The sensitivity of the cell lines was also tested at 30 μM and 10 μM concentration. At 10 μM concentration, betulinic acid did not sensitize any of the cancer cell line. However, many of its derivatives produced concentration dependent growth inhibition effect on several cancer cell lines. Triazole derivatives **5**, **7**, **13**, **15**, **17**, **18** (HL-60 only) and **19** at lowest concentration (10 μM), effected THP-1 and HL-60 the most among all the cell lines tested. Compounds **7**, **13**, **15**, **17**, and **19** were able to cause maximum inhibition (60–65%) of cell proliferation against HL-60 compared to THP-1 where the effect was only 50–55%. For liver (HEP-2) cancer cell line, only two compounds **5** and **20** were able to sensitize the liver cell line at 10 μM, and maximum inhibition of 71% was observed for compound **20**. For lung (A-549) cell line, only three compounds (**5**, **7**, and **17**) showed cytotoxic effects with maximum effect displayed by **7** (60% inhibition). Among prostate (DU-145) and breast (MCF-7) cancer cell lines, baring compound **12** which showed inhibition (70%) against MCF-7, the rest of the compounds failed to exhibit any significant inhibitory effect. In case of CNS cell line, only compound



Scheme 1. Propargylation of betulinic acid **1** and preparation of its 1,2,3-trizole derivatives.

19 showed cytotoxic effect displaying 52% inhibition (at 10 μM concentration). For colon cancer cell line, only compounds **3** and **4** exhibited cytotoxic effect (10 μM conc.) with latter displaying marginally better activity profile (Fig. S1, Table S1).

Though no clear cut structure activity relationship (SAR) could be established, some inferences could still be drawn. Monoalkyloxy (**9**) and hydroxymethyl (**3** and **5**) analogs in general showed better cytotoxic effect than the parent and di- as well as trialkyloxy compounds (**16** and **10**). Among *ortho*- and *para*-cyano (**7** and **8**) isomers, better inhibitory effect was observed for compound **7**. Similar type of effect was also observed for the derivatives bearing phenolic group in the aryl part of the molecules with compound **13** showing maximum inhibitory effects (Table S1). Halogen bearing aryl moiety also showed significant inhibitory effect similar to that of compound **7**.

With the advantage of having more potent compounds than betulinic acid, their IC_{50} values were determined along with betulinic acid (**1**) against cancer cells of different tissue origin (50% growth inhibition) by plotting the graph between concentration *vs* growth inhibition (Table 1).

In leukemia cancer cell lines promising IC_{50} values 2.5, 4.5, 5.5, 5.5, 7, 8, and 8 μM were observed for compounds **7**, **13**, **19**, **15**, **17**, **5**, and **4** respectively against HL-60, similarly promising IC_{50} values 4.5, 8, and 9 μM were also observed for compounds **7**, **13**, and **19** respectively against THP-1. Among all the cell lines, leukemia cells (HL-60 and THP-1) proved most sensitive toward the semi-synthetic analogs in particular compounds **7** and **13** showed maximum cytotoxic effect and were chosen for further detailed study. To verify whether the cancer cell death induced by **7** and **13** was apoptotic, as it has now become 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 [18]. Both compounds were found potent apoptosis inducers, as proved by the measurement of DNA fragmentation. The apoptotic potential of the two cytotoxic agents was further confirmed through the induction of DNA fragmentation in HL-60 cells. Apoptosis typically involves intra-nucleosomal chromatin cleavage by endonucleases in multiples of 180 bp leading to a typical DNA ladder. Both the molecules induced this laddering pattern at a concentration of 20 μM and 30 μM. The laddering pattern was concentration dependent and the minimal concentration inducing DNA fragmentation was observed 20 μM (Fig. 2, Sections 1 and 3). Camptothecin used as a positive control showed fragmentation of DNA in HL-60 cells after treatment of cells with 5 μM camptothecin for 6 h. DNA isolated from untreated and treated normal monkey kidney CV-1 cells did not show any DNA ladder (Fig. 2, Sections 2 and 4) confirming the effect of the molecules specific only for cancer cells sparing the normal cells.

In addition, the effect of **7** and **13** on mitochondrial functioning was also demonstrated and found responsible for the disruption of mitochondrial membrane along with subsequent loss of mitochondrial membrane potential ($\Delta\Psi_m$) in a concentration dependent manner (Fig. 3).

In further study, the clonogenic assay was performed using another leukemia cell line i.e. THP-1 cells which is an adherent cell line so that the colonies could be visualized. Both **7** and **13** produced concentration dependent inhibitory effect on the ability of the cells to reproduce and form large colonies. With respect to the untreated control, the number of colonies formed at lower concentrations of **7** and **13** was comparable but at higher concentration the number of colonies formed was negligible (Fig. 4a and b).

Further *in vitro* cell migration assay was performed in order to analyze the capacity of tumor cells to degrade the extracellular matrix proteins, compounds of the basement membrane and surrounding tissues. Such capacity could be directly correlated with the metastatic potential of tumor cells [19]. Cell cultures treated with **7** and **13** were photographed and cell migration was assessed by comparing the gap sizes between the control and the treatment wells. Representative photomicrographs of tumor cell migration were taken and as depicted in the respective figures, the wound got completely healed in the control wells at the lower concentration of **7** and **13** but at higher concentration the numbers of invasive cells that penetrated the respective wound were inhibited and cell migration got significantly stopped due to the inhibitory effect of the **7** and **13** on cell migration and invasion (Fig. 5a and b). The above study conclusively established the apoptotic character of **7** and **13**. However, kinetic and *in vivo* studies in the preclinical models need to be explored in order to ascertain their therapeutic potency.

Table 1
IC_{50} values in μM of betulinic acid and its analogs on the panel of human cancer cell lines.

Compound	Leukemia		Prostate		Liver	Breast	Lung	CNS	Colon
	THP-1	HL-60	DU-145	PC-3	HEP-2	MCF-7	A-549	SF-295	HCT-15
1	20	17	22	37	16	37	31	22	12
3	15	10	16	49	14	10	14	17	10
4	10	**8**	48	49	23	14	50	44	**8**
5	10	**7**	23	50	**8**	17	32	53	25
6	12	11	23	32	13	12	25	13	46
7	**4.5**	**2.5**	18	14	12	**4.8**	**5**	15	20
8	>50	32	>50	>50	32	49	>50	48	>50
9	47	37	>50	>50	29	12	>50	32	26
10	39	37	>50	>50	>50	10	>50	27	>50
11	22	18	39	>50	>50	27	23	>50	>50
12	18	14	28	13	>50	**3.8**	26	19	26
13	**8**	**3.5**	14	16	13	**9**	16	25	15
14	44	40	50	>50	>50	>50	36	>50	>50
15	50	**5.5**	>50	>50	40	45	>50	>50	28
16	34	30	30	43	26	13	>50	18	50
17	10	**5.5**	34	43	44	15	**8**	>50	13
18	20	**8**	>50	>50	>50	>50	>50	>50	27
19	**9**	**4.5**	24	13	15	38	15	**8**	28
20	46	43	25	39	**3.8**	**8**	>50	44	39

Bold values are shown for those compounds bearing single digit IC_{50} value.

3. Conclusion

The overall results showed high potency of two semi-synthetic derivatives **7** and **13** along with substantial evidence for their apoptotic mode of action against HL-60 and THP-1 cancer cell lines. In addition, the induction of apoptosis by these molecules is evidenced by the loss in mitochondrial membrane potential and appreciable DNA fragmentation. Inhibition of cell migration and inhibitory effect on the ability of the cells to reproduce and form large colonies further substantiated our findings. These studies provide sufficient evidences to advance the compounds (**7** and **13**) for *in vivo* testing in models of human leukemia.

4. Experimental

4.1. Chemistry

All reagents used for chemical synthesis were purchased from Sigma–Aldrich. Solvents used were distilled before use. The progress of all reactions were monitored by TLC on silica gel 60 F_{254} plates (E. Merck) using 2% ceric ammonium sulfate solution for detection of the spots. Purification of the products was carried out by column chromatography. All NMR spectra (^{1}H NMR and ^{13}C

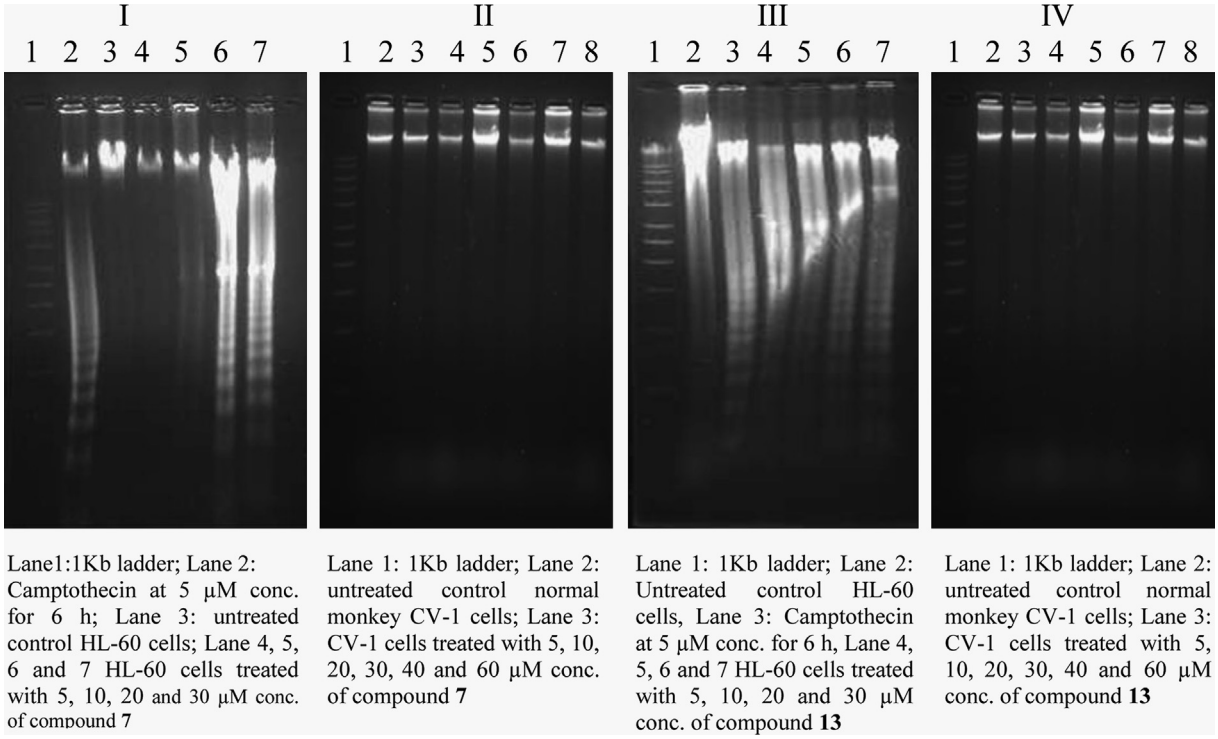


Fig. 2. Compounds **7** and **13** induced DNA fragmentation in HL-60 cells. Cells 2×10^6/mL/well were treated with indicated concentration of **7** and **13** for 24 h. Data are representative of one of two similar experiments. In Sections 1 and 3, HL-60 cells and in Sections 2 and 4, normal monkey CV-1 cells treated with **7** and **13**.

NMR) were recorded on Bruker DPX 200, DPX 400 and DPX 500 instruments using $CDCl_3$ as solvent with TMS as internal standard. Chemical shifts are expressed in δ and coupling constant in Hertz. High resolution mass spectra (HRMS) were recorded on Agilent Technologies 6540 instrument and mass spectra were recorded on ESI-esquire 3000 Bruker Daltonics instrument. IR recorded on an FT-IR Bruker (270-30) spectrophotometer. Melting points (uncorrected) of compounds were recorded on Buchi melting point apparatus B-542.

4.1.1. Isolation of betulinic acid (*1*)

Betulinic acid was isolated in bulk quantity from 90% ethanolic extract of the stem bark of *Platanus orientalis* (duly authenticated by the Taxonomist of our institute). Betulinic acid used in the present study was isolated by repeated column chromatography over silica gel in 15% EtOAc in petroleum ether. Further purification was carried out by crystallization. Purity was obtained >97% which was well characterized by spectroscopic study (data were found in agreement with reported in literature) [8].

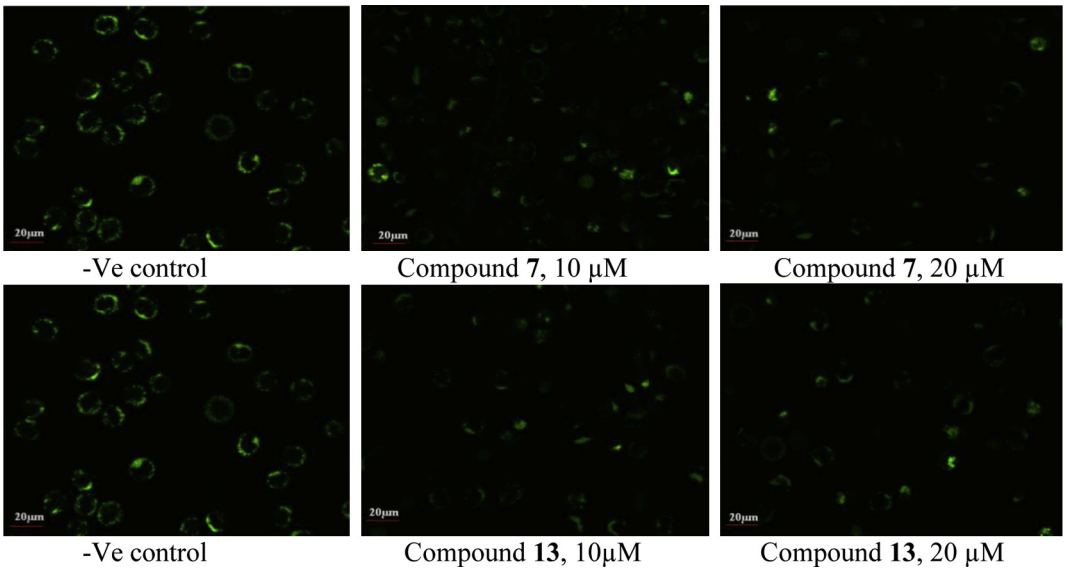


Fig. 3. Compounds **7** and **13** induced loss of mitochondrial membrane potential ($\Delta\Psi_m$) in HL-60. Cells (6×10^4/mL/well) incubated with **7** and **13** at different concentrations in 6 well plates for 24 h. Before 30 min of the completion of the experiment cells were treated with Rodamine-123 (1 μM) in serum free media. Cells were washed with PBS, centrifuged, finally suspended in 1 mL of PBS and analyzed under confocal microscopy. Data are representative of one of two similar experiments.

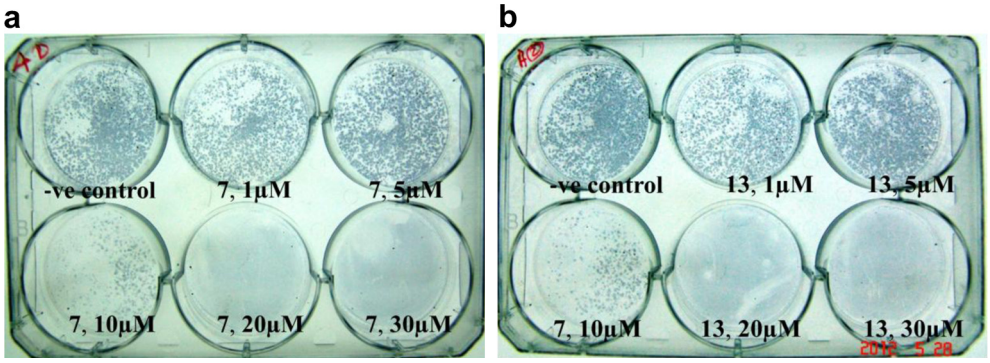


Fig. 4. THP-1 cells were seeded in 6 well plates. After 24 h the cells were treated with indicated concentrations of **7** and **13** for 24 h. After the completion of the treatment the plates were placed in an incubator for a time equivalent to at least six potential cell divisions (to give colonies of >50 cells). After fixation the cells were stained with 0.5% crystal violet for 30 min and allowed to dry at room temperature.

*4.1.2. Preparation of 3-O-propargyl-betulinic acid (**2**)*

The compound prepared by the propargylation of compound **1** (5.0 g, 11 mmol) using the propargyl bromide (2.2 g, 16 mmol) and NaH in dry THF under nitrogen atmosphere for 24 h, after usual work to give the propargylated product **2** colorless solid (4.15 g, 76% yield), mp 223 °C [20]. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.68 (1H, m, *H*-5), 0.75 (3H, s, *H*-25), 0.83 (3H, s, *H*-24), 0.88 and 1.61 (1H each, m, *H*-1), 0.93 (3H, s, *H*-26), 0.97 (6H, s, *H*-27 and *H*-23), 1.17 and 2.43 (1H each, m, *H*-11), 1.21 and 1.99 (1H each, m, *H*-12), 1.27 and 1.94 (1H each, m, *H*-15), 1.34 and 1.51 (1H each, m, *H*-6), 1.36 and 1.41 (1H each, m, *H*-7), 1.37 and 2.14 (1H each, m, *H*-21), 1.38 (1H, m, *H*-9), 1.47 and 2.20 (1H each, m, *H*-22), 1.46 and 2.25 (1H each, m, *H*-16), 1.61 (1H, m, *H*-18), 1.69 (3H, s, *H*-30), 1.74 (2H, m, *H*-2), 2.31 (1H, m, *H*-13), 2.35 (1H, s, $-OCH_2CCH-$), 2.98 (2H, m, H-3, *H*-19), 4.20 (2H, m, $-OCH_2-$), 4.61 and 4.74 (1H each, s, *H*-29). ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.10, 150.43, 109.69, 85.88, 80.96, 73.42, 56.42, 56.42, 55.93, 50.49, 49.31, 46.93, 42.43, 40.74, 38.61, 38.57, 38.44, 37.17, 37.06, 34.34, 32.18, 30.60, 29.70, 28.00, 25.53, 22.69, 20.89, 19.40, 18.22, 16.17, 16.10, 16.04, 14.12. IR γ_{max} (neat): 3434, 3310, 2942, 2868, 1713, 1453, 1317, 1149, 1123, 1043, 1009, 983, 885 cm^{-1}. MS (%) for $C_{33}H_{50}O_3$ at *m*/*z* 495.7497 $[M + 1]^+$ 495 (19.3), 477 (5.3), 429 (4.3), 379 (29.0), 360 (22.5), 341 (24.7), 317 (67.7), 350 (21.0), 299/300 (21.0), 279 (100), 250 (15), 228 (19), 205 (24), 202 (34), 148 (23.6), 110 (19.6), 80 (15). HRMS *m*/*z* calcd. for $C_{33}H_{50}O_3$ $[M + H]^+$ 495.38327, found 495.38213.

4.1.3. General procedure for synthesis of aromatic azides

The aromatic azides are prepared (Scheme 2) by addition of 6 N HCl solution to stirring dichloromethane solution of the appropriate amine at ≤0 °C followed by drop wise saturated aqueous solution of $NaNO_2$ and the contents stirred for 30 min. To the reaction mixture, added NaN_3 stirred the contents for further 30 min. The contents were allowed to attain room temperature, the two phases were separated, and the aqueous phase extracted with DCM. The combined organic layers were washed with aqueous solution of $NaHCO_3$, followed by then brine solution, dried over sodium sulfate, filtered and concentrated under reduced pressure to give the aryl azides which were used in the next step without further purification.

4.1.4. General procedure for synthesis of 2,3,4,6-tetra acetyl sugar azides

2,3,4,6-Tetra acetyl sugar azides were prepared (Scheme 2) by dissolving acetylated sugar in DCM and HBr in acetic acid was added drop wise with stirring under inert conditions at 0 °C. The mixture was stirred for another 15 min and on usual workup followed by evaporation yellow syrup was obtained. This syrup was dissolved in dry DMF and to the stirring mixture NaN_3 was added. The mixture was stirred at room temperature for 3 h. On completion, the reaction was worked up by usual method to give the azide product. Further purification was carried out by re-crystallization in methanol to yield pure azides.

*4.1.5. General experimental procedure for preparation 1,2,3-triazoles of betulinic acid (**3**–**20**)*

The title compounds were prepared by the reaction of substituted aryl and sugar azide (say 1 equiv), 3-*O*-propargyl-betulinic acid (≥1 equiv), $CuSO_4$ (0.1 equiv) in *t*-BuOH:water (1:1) mixture. Sodium ascorbate (0.5 equiv) was added to the reaction mixture and the contents stirred vigorously at room temperature for 2–8 h till completion of the reaction (monitored by TLC analysis) [20]. The reaction was worked up by dilution of the contents with water and extraction with ethyl acetate (3 times). The combined ethyl acetate extract was washed with brine solution, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure on a rotavapor. The crude product obtained thus subjected was put to column chromatography (silica gel) with EtOAc:Hexane (1:4) mixture as eluent to afford the desired pure products in 92–98% yields.

*4.1.5.1. Synthesis of 3{1N(2-hydroxymethyl phenyl)-1H-1,2,3-triazol-4yl}methyloxy betulinic acid (**3**).* The title compound prepared by the reaction of propargylated betulinic acid (100 mg, 0.2 mmol) and 1-azido-2-hydroxymethyl benzene (40 mg, 0.25 mmol) in *t*-BuOH:water (1:1) mixture (5 mL) as per the method described in Section 4.1.5 to furnish **3** colorless solid (117 mg, 97% yield), mp 215 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.65 (1H, m, *H*-5), 0.70 (3H, s, *H*-25), 0.73 (3H, s, *H*-24), 0.74 (3H, s, *H*-26), 0.88 and 1.50 (1H each, m, *H*-1), 0.94 (6H, s, *H*-23 and *H*-27), 1.13 and 1.85 (1H each, m, *H*-11), 1.21 and 1.59 (1H each, m, *H*-12), 1.17 and 1.45 (1H each, m, *H*-15), 1.24 and 1.50 (1H each, m, *H*-6), 1.26 and 1.41 (1H each, m, *H*-7), 1.27 and 1.68 (1H each, m, *H*-21), 1.18 (1H, m, *H*-9), 1.47 and 1.86 (1H each, m, *H*-22), 1.46 and 2.15 (1H each, m, *H*-16), 1.49 (1H, m, *H*-18), 1.54 (2H, m, *H*-2), 1.69 (3H, s, *H*-30), 2.30 (1H, m, *H*-13), 2.96 (1H, m, *H*-19), 3.16 (1H, m, *H*-3), 4.47 (2H, s, $-CH_2OH$), 4.61 and 4.73 (1H each, s, H-29), 5.29 and 5.36 (1H each, d, J = 12.1 Hz, $-OCH_2-$), 7.38 (1H, d, J = 8.10 Hz, Ar–*H*), 7.48–7.53 (2H, m, Ar–*H*), 7.65 (1H, d, J = 8.11 Hz, Ar–*H*), 8.04 (1H, s, N–C*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.18, 150.37, 143.56, 135.72, 135.56, 131.55, 130.16, 129.12, 125.52, 124.37, 109.78, 78.91, 61.69, 56.93, 56.59, 55.26, 50.43, 49.40, 46.96, 42.33, 40.63, 38.83, 38.67, 38.29, 37.12, 36.91, 34.18, 31.95, 30.53, 29.60, 27.97, 27.31, 25.44, 20.82, 19.34, 18.20, 16.06, 15.81, 15.38, 14.68. IR γ_{max} (neat): 3413, 2938, 2856, 1720, 1638, 1617, 1465,

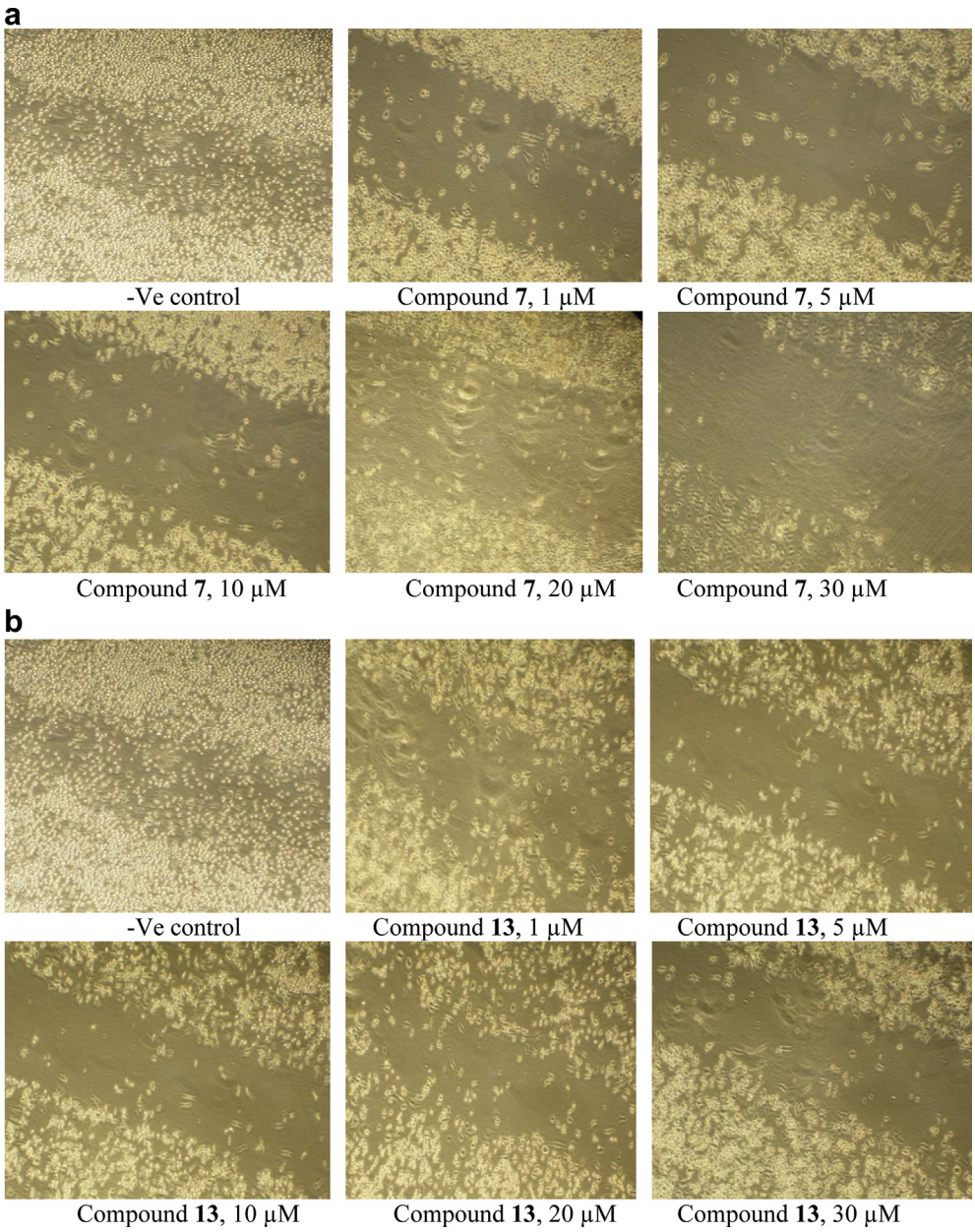


Fig. 5. a. THP-1 cells were seeded in six wells plate. When a tight cell monolayer has formed a wound was created using a 1000 μL tip, cells were then treated with indicated concentrations of compound **7**. The number of cells that migrated toward the wound after the treatment was visualized using 10× objective Olympus. b. THP-1 cells were seeded in six wells plate. When a tight cell monolayer has formed a wound was created using a 1000 μL tip, cells were then treated with indicated concentrations of compound **13**. The number of cells that migrated toward the wound after the treatment was visualized using 10× objective Olympus.

1376, 1244, 1165, 1131, 1099, 1061, 1011, 886, 759 cm^{-1}. MS (%) for $C_{40}H_{57}N_3O_4$ at *m*/*z* 666.9045 $[M + Na]^+$, 667 (7), 644 (100), 626 (17.3), 612 (50), 595 (19.4), 571 (16.6), 554 (52.7), 545 (100), 539 (52.7), 452 (15), 201 (43), 102 (38). HRMS *m*/*z* calcd. for $C_{40}H_{57}N_3O_4$ $[M + H]^+$ 644.4422, found 644.4388.

*4.1.5.2. Synthesis of 3{1N(4-methoxyphenyl)-1H-1,2,3-triazol-4yl} methyloxy betulinic acid (**4**).* The title compound prepared by the reaction of propargylated betulinic acid (115 mg, 0.23 mmol) and 1-azido-4-methoxy benzene (45 mg, 0.3 mmol) as per the method described in Section 4.1.5 to furnish **4** colorless solid (145 mg, 98% yield), mp 238 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.62 (1H, m, *H*-5), 0.64 (3H, s, *H*-25), 0.72 (3H, s, *H*-24), 0.73 (3H, s, *H*-26), 0.87 and 1.51 (1H each, m, *H*-1), 0.90 and 92 (3H each, s, *H*-23 and *H*-27), 1.13 and 1.87 (1H each, m, *H*-11), 1.21 and 1.59 (1H each, m, *H*-12), 1.17 and 1.45 (1H each, m, *H*-15), 1.24 and 1.52 (1H each, m, *H*-6), 1.26 and 1.41 (1H each, m, *H*-7), 1.27 and 1.67 (1H each, m, *H*-21), 1.18 (1H, m, *H*-9), 1.47 and 1.89 (1H each, m, *H*-22), 1.46 and 2.12 (1H each, m, *H*-16), 1.51 (1H, m, *H*-18), 1.55 (2H, m, *H*-2), 1.69 (3H, s, *H*-30), 2.29 (1H, m, *H*-13), 3.02 (1H, m, *H*-19), 3.16 (1H, m, *H*-3), 3.87 (3H, s, $-OCH_3$), 4.60 and 4.73 (1H each, s, *H*-29), 5.25 and 5.34 (1H each, d, J = 16.0 Hz, $-OCH_2-$), 7.03 (2H, d, J = 8.0 Hz, Ar–*H*), 7.62 (2H, d, J = 8.0 Hz, Ar–*H*), 7.99 (1H, s, N–C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.20, 159.93, 150.42, 143.70, 130.38, 122.57, 122.22, 114.76, 109.67, 78.92, 57.09, 56.55, 55.66, 55.29, 50.47, 49.51, 47.04, 42.33, 40.63, 38.83, 38.68, 38.37, 37.14, 36.87, 34.18, 31.99, 30.60, 29.71, 27.96, 27.36, 22.69, 20.85, 19.37, 18.21, 16.03, 16.01, 16.0, 14.65. IR γ_{max} (neat): 3472, 2936, 2872, 1707, 1636, 1615,

1518, 1451, 1388, 1259, 1171, 1109, 1054, 1030, 834, 760 cm^{-1}. MS (%) for $C_{40}H_{57}N_3O_4$ at *m/z* 644.9252 $[M + H]^+$, 644 (100), 601 (11.5), 541 (7.6), 473 (18.4), 387 (13), 339 (17), 233 (16.9), 451 (26), 282 (46), 102 (13.8). HRMS *m/z* calcd. for $C_{40}H_{57}N_3O_4$ $[M + H]^+$ 644.4422, found 644.4387.

*4.1.5.3. Synthesis of 3{1N(3-hydroxymethylphenyl)-1H-1,2,3-triazol-4yl}methyloxy betulinic acid (**5**).* The title compound prepared by the reaction of propargylated betulinic acid (125 mg, 0.25 mmol) and 1-azido-3-hydroxymethyl benzene (45 mg, 0.3 mmol) as per the method described in Section 4.1.5 to furnish **5** (151 mg, 94% yield), mp 250–252 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.61 (1H, m, *H*-5), 0.63 (3H, s, *H*-25), 0.70 (3H, s, *H*-24), 0.71 (3H, s, *H*-26), 0.87 and 1.50 (1H each, m, *H*-1), 0.92 and 0.93 (3H each, s, *H*-23 and *H*-27), 1.09 and 1.85 (1H each, m, *H*-11), 1.21 and 1.59 (1H each, m, *H*-12), 1.16 and 1.45 (1H each, m, *H*-15), 1.24 and 1.50 (1H each, m, *H*-6), 1.26 and 1.38 (1H each, m, *H*-7), 1.27 and 1.64 (1H each, m, *H*-21), 1.20 (1H, m, *H*-9), 1.47 and 1.86 (1H each, m, *H*-22), 1.46 and 2.09 (1H each, m, *H*-16), 1.49 (1H, m, *H*-18), 1.54 (2H, m, *H*-2), 1.67 (3H, s, *H*-30), 2.28 (1H, m, *H*-13), 3.01 (1H, m, *H*-19), 3.17 (1H, m, *H*-3), 4.60 and 4.73 (1H each, s, *H*-29), 4.80 (2H, s, $-CH_2OH$), 5.28 and 5.35 (1H each, d, *J* = 12.1 Hz, $-OCH_2-$), 7.44 (1H, d, *J* = 8.10 Hz, Ar–*H*), 7.50 (1H, dd, *J* = 8.10, 8.0 Hz, Ar–*H*), 7.63 (1H, d, *J* = 8.0 Hz, Ar–*H*), 7.77 (1H, s, Ar–*H*), 8.11 (1H, s, N–C*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.23, 150.39, 143.81, 143.43, 136.95, 129.82, 127.09, 122.65, 119.43, 119.22, 109.74, 78.96, 64.15, 56.95, 56.55, 55.23, 50.41, 49.46, 46.98, 42.30, 40.59, 38.80, 38.65, 38.34, 37.09, 36.86, 34.13, 31.96, 30.55, 29.72, 27.26, 25.45, 22.72, 20.81, 19.38, 18.12, 16.01, 15.64, 15.39, 14.67. IR γ_{max} (neat): 3457, 3315, 2929, 2869, 1714, 1687, 1639, 1618, 1461, 1376, 1236, 1107, 1099, 1041, 975, 881, 795 cm^{-1}. MS (%) for $C_{40}H_{57}N_3O_4$ at *m/z* 666.9448 $[M + Na]^+$, 667 (80), 644 (77.4), 626 (48.3), 602 (20.9), 598 (25.8), 594 (41.9), 578 (41.9), 566 (19.3), 556 (83.8), 549 (100), 400 (34.9), 398 (41.2), 324 (68.2), 254 (30), 207 (28), 154 (25.3), 112 (15.8). HRMS *m/z* calcd. for $C_{40}H_{57}N_3O_4$ $[M + H]^+$ 644.4422, found 644.4383.

*4.1.5.4. Synthesis of 3{1N(4-cyanomethylphenyl)-1H-1,2,3-triazol-4yl}methyloxy betulinic acid (**6**).* The title compound prepared by the reaction of propargylated betulinic acid (100 mg, 0.2 mmol) and 1-azido-4-cyanomethyl benzene (40 mg, 0.25 mmol) as per the method described in Section 4.1.5 to furnish **6** (127 mg, 97% yield), mp 202–203 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.61 (3H, s, *H*-25), 0.63 (1H, m, *H*-5), 0.72 (3H, s, *H*-24), 0.73 (3H, s, *H*-26), 0.89 and 1.50 (1H each, m, *H*-1), 0.93 and 0.94 (3H each, s, *H*-23 and *H*-27), 1.09 and 1.85 (1H each, m, *H*-11), 1.21 and 1.59 (1H each, m, *H*-12), 1.16 and 1.45 (1H each, m, *H*-15), 1.24 and 1.50 (1H each, m, *H*-6), 1.26 and 1.38 (1H each, m, *H*-7), 1.27 and 1.64 (1H each, m, *H*-21), 1.20 (1H, m, *H*-9), 1.47 and 1.86 (1H each, m, *H*-22), 1.46 and 2.17 (1H each, m, *H*-16), 1.49 (1H, m, *H*-18), 1.54 (2H, m, *H*-2), 1.69 (3H, s, *H*-30), 2.27 (1H, m, *H*-13), 3.03 (1H, m, *H*-19), 3.19 (1H, m, *H*-3), 3.67 (2H, s, $-CH_2CN$), 4.60 and 4.74 (1H each, s, *H*-29), 5.28 and 5.37 (1H each, d, *J* = 12.0 Hz, $-OCH_2-$), 7.85 (2H, d, *J* = 8.0 Hz, Ar–*H*), 7.97 (2H, d, *J* = 8.0 Hz, Ar–*H*), 8.17 (1H, s, N–C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.35, 150.30, 144.26, 135.33, 130.21, 129.43, 127.60, 122.38, 120.17, 120.73, 109.77, 79.23, 60.50, 56.83, 56.61, 55.26, 50.43, 49.51, 47.01, 42.34, 40.63, 38.82, 38.66, 38.42, 37.12, 36.88, 34.17, 31.98, 31.59, 29.69, 27.95, 27.21, 25.48, 22.69, 20.97, 20.07, 19.34, 16.31, 16.23, 16.16, 14.83. IR γ_{max} (neat): 3387, 3213, 2930, 2869, 2245, 1716, 1617, 1463, 1376, 1244, 1165, 1131, 1109, 1042, 881, 806 cm^{-1}. MS (%) for $C_{41}H_{56}N_4O_3$ at *m/z* 675.4389 $[M + Na]^+$, 675 (12), 636 (43.7), 617 (26.5), 599 (39.5), 553 (39), 488 (45.2), 461 (35.9), 442 (54.6), 416 (84.3), 387 (93.7), 348 (100), 282 (39.0), 174 (17). HRMS *m/z* calcd. for $C_{41}H_{56}N_4O_3$ $[M + H]^+$ 653.4425, found 653.4403.

*4.1.5.5. Synthesis of 3{1N(2-cyanophenyl)-1H-1,2,3-triazol-4yl} methyloxy betulinic acid (**7**).* The title compound prepared by the reaction of propargylated betulinic acid (100 mg, 0.2 mmol) and 2-azido benzonitrile (40 mg, 0.27 mmol) as per the method described in Section 4.1.5 to furnish **7** (120 mg, 95% yield), mp 243–245 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.65 (1H, m, *H*-5), 0.69 (3H, s, *H*-25), 0.73 (3H, s, *H*-24), 0.74 (3H, s, *H*-26), 0.88 and 1.50 (1H each, m, *H*-1), 0.94 and 0.95 (3H each, s, *H*-23 and *H*-27), 1.09 and 1.85 (1H each, m, *H*-11), 1.21 and 1.59 (1H each, m, *H*-12), 1.16 and 1.45 (1H each, m, *H*-15), 1.24 and 1.50 (1H each, m, *H*-6), 1.26 and 1.38 (1H each, m, *H*-7), 1.27 and 1.64 (1H each, m, *H*-21), 1.20 (1H, m, *H*-9), 1.47 and 1.86 (1H each, m, *H*-22), 1.46 and 2.14 (1H each, m, *H*-16), 1.49 (1H, m, *H*-18), 1.54 (2H, m, *H*-2), 1.69 (3H, s, *H*-30), 2.29 (1H, m, *H*-13), 3.01 (1H, m, *H*-19), 3.17 (1H, m, *H*-3), 4.59 and 4.73 (1H each, s, *H*-29), 5.31 and 5.36 (1H each, d, *J* = 12.0 Hz, $-OCH_2-$), 7.64 (1H, d, *J* = 8.0 Hz, Ar–*H*), 7.82–7.89 (3H, m, Ar–*H*), 8.31 (1H, s, N–C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.99, 150.43, 144.14, 138.40, 134.41, 134.28, 129.67, 125.42, 129.53, 115.51, 109.66, 106.73, 78.93, 56.81, 56.59, 55.28, 50.48, 49.50, 46.93, 42.34, 40.67, 38.84, 38.68, 38.34, 37.15, 37.87, 34.20, 31.99, 30.58, 29.64, 27.96, 27.36, 25.48, 20.82, 19.38, 18.23, 16.04, 15.74, 15.37, 14.68. IR γ_{max} (neat): 3506, 3401, 2945, 2870, 2231, 1719, 1638, 1609, 1463, 1376, 1249, 1175, 1109, 1042, 882, 843, 618 cm^{-1}. MS (%) for $C_{40}H_{54}N_4O_3$ at *m/z* 637.4305 $[M - H]^+$, 637 (18.9), 601 (18), 505 (23.8), 403 (28), 323 (100), 310 (85.6), 252 (62.4), 241 (85.6). HRMS *m/z* calcd. for $C_{40}H_{54}N_4O_3$ $[M + H]^+$ 639.4268, found 639.4269.

*4.1.5.6. Synthesis of 3{1N(4-cyanophenyl)-1H-1,2,3-triazol-4yl}methyloxy betulinic acid (**8**).* The title compound prepared by the reaction of propargylated betulinic acid (75 mg, 0.15 mmol) and 4-azido benzonitrile (30 mg, 0.2 mmol) as per the method described in Section 4.1.5 to furnish **8** (90 mg, 94% yield), mp 231–233 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.62 (3H, s, *H*-25), 0.64 (1H, m, *H*-5), 0.71 (3H, s, *H*-24), 0.74 (3H, s, *H*-26), 0.88 and 1.50 (1H each, m, *H*-1), 0.94 and 0.95 (3H each, s, *H*-23 and *H*-27), 1.09 and 1.85 (1H each, m, *H*-11), 1.21 and 1.59 (1H each, m, *H*-12), 1.16 and 1.45 (1H each, m, *H*-15), 1.24 and 1.50 (1H each, m, *H*-6), 1.26 and 1.38 (1H each, m, *H*-7), 1.27 and 1.64 (1H each, m, *H*-21), 1.20 (1H, m, *H*-9), 1.47 and 1.86 (1H each, m, *H*-22), 1.46 and 2.15 (1H each, m, *H*-16), 1.49 (1H, m, *H*-18), 1.54 (2H, m, *H*-2),1.69 (3H, s, *H*-30), 2.28 (1H, m, *H*-13), 3.02 (1H, m, *H*-19), 3.19 (1H, m, *H*-3), 4.62 and 4.74 (1H each, s, *H*-29), 5.27 and 5.36 (1H each, d, *J* = 12.0 Hz, $-OCH_2-$), 7.83 (1H, d, *J* = 8.0 Hz, Ar–*H*), 7.93 (2H, d, *J* = 8.0 Hz, Ar–*H*), 8.11 (1H, s, N–C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.92, 150.31, 144.66, 139.66, 133.96, 122.27, 120.60, 117.67, 112.60, 109.81, 78.91, 56.81, 56.57, 55.23, 50.40, 49.45, 47.01, 42.32, 40.61, 38.83, 38.65, 38.40, 37.11, 36.85, 34.14, 31.94, 30.55, 29.72, 27.95, 27.33, 25.44, 20.82, 19.34, 18.19, 16.03, 15.60, 15.39, 14.69. IR γ_{max} (neat): 3470, 3230, 2946, 2872, 2232, 1707, 1636, 1611, 1465, 1378, 1248, 1176, 1110, 1043, 885, 845, 619 cm^{-1}. MS (%) for $C_{40}H_{54}N_4O_3$ at *m/z* 661.4311 $[M + Na]^+$, 661 (41.66), 638 (16.6), 614 (4), 506 (20.8), 404 (25), 324 (100), 311 (91.6), 253 (66.6), 242 (83.3). HRMS *m/z* calcd. for $C_{40}H_{54}N_4O_3$ $[M + H]^+$ 639.4268, found 639.4262.

*4.1.5.7. Synthesis of 3{1N(3-methoxyphenyl)-1H-1,2,3-triazol-4yl} methyloxy betulinic acid (**9**).* The title compound prepared by the reaction of propargylated betulinic acid (125 mg, 0.25 mmol) and 1-azido-3-methoxy benzene (45 mg, 0.3 mmol) as per the method described in Section 4.1.5 to furnish **9** (155 mg, 96% yield), mp 233 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.62 (1H, m, *H*-5), 0.63 (3H, s, *H*-25), 0.71 (3H, s, *H*-24), 0.72 (3H, s, *H*-26), 0.88 and 1.50 (1H each, m, *H*-1), 0.92 and 0.94 (3H each, s, *H*-23 and *H*-27), 1.09 and 1.85 (1H each, m, *H*-11), 1.21 and 1.59 (1H each, m, *H*-12), 1.16 and 1.45 (1H each, m, *H*-15), 1.24 and 1.50 (1H each, m, *H*-6), 1.26 and 1.38 (1H each, m, *H*-7), 1.27 and 1.64 (1H each, m, *H*-21), 1.20 (1H, m, *H*-

9), 1.47 and 1.86 (1H each, m, *H*-22), 1.46 and 2.09 (1H each, m, *H*-16), 1.49 (1H, m, *H*-18), 1.54 (2H, m, *H*-2), 1.70 (3H, s, *H*-30), 2.28 (1H, m, *H*-13), 3.03 (1H, m, *H*-19), 3.17 (1H, m, *H*-3), 3.89 (3H, s, −OC*H*$_3$), 4.60 and 4.74 (1H each, s, *H*-29), 5.25 and 5.36 (1H each, d, J = 12.0 Hz, −OC*H*$_2$−), 6.98 (1H, d, J = 8.01 Hz, Ar−*H*), 7.26 (1H, d, J = 8.0 Hz, Ar−*H*), 7.33 (1H, s, Ar−*H*), 7.42 (1H, dd, J = 8.0, 8.01 Hz, Ar−*H*), 8.08 (1H, s, N−C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.24, 160.61, 150.43, 143.86, 137.90, 130.54, 122.60, 114.74, 112.44, 109.71, 106.43, 78.95, 57.02, 56.65, 56.56, 55.26, 50.44, 49.48, 47.00, 42.31, 40.61, 38.83, 38.66, 38.35, 37.12, 36.87, 34.15, 31.98, 30.58, 29.68, 27.96, 27.35, 25.47, 20.83, 19.38, 18.19, 16.00, 15.68, 15.35, 14.67. IR γ_{max} (neat): 3514, 3335, 2942, 2869, 1721, 1631, 1611, 1462, 1376, 1256, 1157, 1107, 1086, 1042, 855, 776, 684 cm^{-1}. MS (%) for $C_{40}H_{57}N_3O_4$ at m/z 643.4449 [M − H]$^+$, 643 (53.4), 576 (17), 526 (36.9), 519.9 (17.8), 486 (10.2), 453 (17.6), 439 (17.8), 429.7 (54.7), 377 (31.5), 365 (31.5), 347.9 (23.2), 339 (35.6), 325 (58.9), 313 (64.3), 278 (72.6), 226 (34.4), 256 (34.2), 248 (63), 226 (31.5), 174 (69.8), 112 (37.6), 278 (72.6), 248 (64.3). HRMS m/z calcd. for $C_{40}H_{57}N_3O_4$ [M + H]$^+$ 644.4422, found 639.4390.

*4.1.5.8. Synthesis of 3{1N(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-4yl}methyloxy betulinic acid (**10**).* The title compound prepared by the reaction of propargylated betulinic acid (120 mg, 0.24 mmol) and 1-azido-3,4,5-methoxy benzene (52 mg, 0.3 mmol) as per the method described in Section 4.1.5 to furnish **10** (155 mg, 92% yield), mp 221–223 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.62 (1H, m, *H*-5), 0.63 (3H, s, *H*-25), 0.71 (3H, s, *H*-24), 0.72 (3H, s, *H*-26), 0.88 and 1.50 (1H each, m, *H*-1), 0.93 and 0.94 (3H each, s, *H*-23 and *H*-27), 1.09 and 1.85 (1H each, m, *H*-11), 1.21 and 1.59 (1H each, m, *H*-12), 1.16 and 1.45 (1H each, m, *H*-15), 1.24 and 1.50 (1H each, m, *H*-6), 1.26 and 1.38 (1H each, m, *H*-7), 1.27 and 1.64 (1H each, m, *H*-21), 1.20 (1H, m, *H*-9), 1.47 and 1.86 (1H each, m, *H*-22), 1.46 and 2.06 (1H each, m, *H*-16), 1.49 (1H, m, *H*-18), 1.54 (2H, m, *H*-2), 1.70 (3H, s, *H*-30), 2.28 (1H, m, *H*-13), 3.03 (1H, m, *H*-19), 3.18 (1H, m, *H*-3), 3.89 (6H, s, 2× −OC*H*$_3$), 3.82 (3H, s, −OC*H*$_3$), 4.60 and 4.74 (1H each, s, *H*-29), 5.25 and 5.36 (1H each, d, J = 12.0 Hz, −OC*H*$_2$−), 6.93 (2H, s, Ar−*H*), 8.08 (1H, s, N−C*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.36, 153.89 (2× C), 150.34, 143.75, 138.29, 132.73, 122.62, 109.74, 98.31 (2× CH), 78.96, 61.07, 56.64, 56.54, 56.12 (2× OCH_3), 55.21, 50.37, 49.42, 47.01, 42.30, 40.59, 38.80, 38.63, 38.33, 37.09, 36.85, 34.12, 31.95, 30.55, 29.70, 28.42, 27.28, 25.76, 20.82, 19.34, 18.20, 16.07, 15.60, 15.35, 14.66. IR γ_{max} (neat): 3468, 3236, 2940, 2869, 1715, 1623, 1615, 1512, 1472, 1376, 1233, 1129, 1042, 1007.5, 881, 828, 618 cm^{-1}. MS (%) for $C_{42}H_{61}N_3O_6$ at m/z 702.4517 [M − H]$^+$, 702 (100), 657 (57.1), 611 (53.9), 551 (57.1), 490 (87.3), 464 (69.8), 417 (76.1), 412 (71.4), 315 (50.7), 264 (36.5), 204 (41.2), 152 (58.7). HRMS m/z calcd. for $C_{42}H_{61}N_3O_6$ [M + H]$^+$ 704.4633, found 704.4593.

*4.1.5.9. Synthesis of 3{1N(5-iodo-2-methylphenyl)-1H-1,2,3-triazol-4yl}methyloxy betulinic acid (**11**).* The title compound prepared by the reaction of propargylated betulinic acid (85 mg, 0.17 mmol) and 2-azido-4-iodo-1-methylbenzene (55 mg, >0.2 mmol) as per the method described in Section 4.1.5 to furnish **11** (122 mg, 96% yield), mp 230–233 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.65 (1H, m, *H*-5), 0.69 (3H, s, *H*-25), 0.74 (3H, s, *H*-24), 0.79 (3H, s, *H*-26), 0.88 and 1.50 (1H each, m, *H*-1), 0.94 and 0.95 (3H each, s, *H*-23 and *H*-27), 1.09 and 1.85 (1H each, m, *H*-11), 1.21 and 1.59 (1H each, m, *H*-12), 1.16 and 1.45 (1H each, m, *H*-15), 1.24 and 1.50 (1H each, m, *H*-6), 1.26 and 1.38 (1H each, m, *H*-7), 1.27 and 1.64 (1H each, m, *H*-21), 1.20 (1H, m, *H*-9), 1.47 and 1.86 (1H each, m, *H*-22), 1.46 and 2.09 (1H each, m, *H*-16), 1.49 (1H, m, *H*-18), 1.54 (2H, m, *H*-2), 1.70 (3H, s, *H*-30), 2.17 (3H, s, Ar−C*H*$_3$), 2.28 (1H, m, *H*-13), 3.01 (1H, m, *H*-19), 3.17 (1H, m, *H*-3), 4.60 and 4.73 (1H each, s, *H*-29), 5.25 and 5.37 (1H each, d, J = 12.0 Hz, −OC*H*$_2$−), 7.12 (1H, d, J = 8.0 Hz, Ar−*H*), 7.67 (1H, d, J = 1.80 Hz, Ar−*H*), 7.74 (1H, dd, J = 8.0, 1.80 Hz, Ar−*H*), 7.85 (1H, s, N−C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.17, 150.34, 143.25, 138.87, 137.24, 134.54, 133.26, 133.05, 125.66, 109.71, 90.08, 78.91, 56.91, 56.57, 55.34, 50.46, 49.44, 46.95, 42.33, 40.70, 38.85, 38.70, 38.31, 37.17, 36.91, 34.23, 31.95, 30.55, 29.60, 27.99, 27.37, 25.49, 20.85, 19.38, 18.33, 17.68, 16.35, 15.85, 15.45, 14.68. IR γ_{max} (neat): 3345, 3221, 2992, 2868, 1711, 1617, 1463, 1376, 1244, 1192, 1133, 1077, 1039, 941, 883, 810, 620 cm^{-1}. MS (%) for $C_{40}H_{56}IN_3O_3$ at m/z 752.3531 [M − H]$^+$, 752 (100), 725 (4.2), 670 (9.1), 645 (29.5), 610 (7.7), 580 (21.1), 567 (23.9), 518 (25.3), 493 (90), 455 (55.6), 374 (28.1), 340 (19.7), 327 (73.2), 310 (71.8), 282 (97.1), 265 (32.3), 254 (76.0), 235 (57.7), 198 (59.1), 134 (25.5). HRMS m/z calcd. for $C_{40}H_{56}IN_3O_3$ [M + H]$^+$ 754.3439, found 754.3389.

*4.1.5.10. Synthesis of 3{1N(3-methyl-5-hydroxyphenyl)-1H-1,2,3-triazol-4yl}methyloxy betulinic acid (**12**).* The title compound prepared by the reaction of propargylated betulinic acid (100 mg, 0.20 mmol) and 3-azido-5-methylphenol (45 mg, 0.3 mmol) as per the method described in Section 4.1.5 to furnish **12** (121 mg, 94% yield), mp 267–270 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.64 (1H, m, *H*-5), 0.67 (3H, s, *H*-25), 0.72 (3H, s, *H*-24), 0.73 (3H, s, *H*-26), 0.89 and 1.51 (1H each, m, *H*-1), 0.93 and 0.95 (3H each, s, *H*-23 and *H*-27), 1.09 and 1.84 (1H each, m, *H*-11), 1.21 and 1.59 (1H each, m, *H*-12), 1.16 and 1.45 (1H each, m, *H*-15), 1.24 and 1.50 (1H each, m, *H*-6), 1.26 and 1.38 (1H each, m, *H*-7), 1.27 and 1.64 (1H each, m, *H*-21), 1.20 (1H, m, *H*-9), 1.47 and 1.86 (1H each, m, *H*-22), 1.46 and 2.09 (1H each, m, *H*-16), 1.49 (1H, m, *H*-18), 1.54 (2H, m, *H*-2), 1.69 (3H, s, *H*-30), 2.13 (3H, s, Ar−C*H*$_3$), 2.26 (1H, m, *H*-13), 3.01 (1H, m, *H*-19), 3.16 (1H, m, *H*-3), 4.61 and 4.75 (1H each, s, *H*-29), 5.25 and 5.36 (1H each, d, J = 12.0 Hz, −OC*H*$_2$−), 7.01 (1H, s, Ar−*H*), 7.18 (1H, s, Ar−*H*), 7.33 (1H, s, Ar−*H*), 8.08 (1H, s, N−C*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 179.63, 154.53, 151.54, 147.25, 138.60, 130.23, 126.68, 124.08, 120.36, 118.24, 109.68, 86.75, 56.62, 55.99, 55.77, 50.48, 49.29, 46.90, 43.26, 40.07, 38.54, 38.47, 38.39, 37.42, 37.15, 34.32, 32.41, 30.48, 29.59, 28.09, 27.19, 25.51, 22.96, 20.89, 19.35, 18.12, 16.26, 16.15, 16.07, 14.68. IR γ_{max} (neat): 3544, 2993, 2871, 1699, 1619, 1465, 1377, 1246, 1195, 1131, 1097, 1009, 945, 891, 868, 723 cm^{-1}. MS (%) for $C_{40}H_{57}N_3O_4$ at m/z 644.9847 [M + H]$^+$, 644 (6.4), 621 (65.6), 561 (66.0), 621 (65.6), 536 (51.5), 501 (48.4), 471 (100), 414 (65.6), 356 (56.2), 324 (31.2), 28 (56.2), 208 (34.3), 178 (37.5). HRMS m/z calcd. for $C_{40}H_{57}N_3O_4$ [M + H]$^+$ 644.4422, found 644.4393.

*4.1.5.11. Synthesis of 3{1N(5-hydroxy-naphth-1yl)-1H-1,2,3-triazol-4yl}methyloxy betulinic acid (**13**).* The title compound prepared by the reaction of propargylated betulinic acid (75 mg, 0.15 mmol) and 5-azidonaphthalen-1-ol (37 mg, 0.2 mmol) as per the method described in Section 4.1.5 to furnish **13** (98 mg, 97% yield), mp 238–240 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.63 (1H, m, *H*-5), 0.68 (3H, s, *H*-25), 0.70 (3H, s, *H*-24), 0.73 (3H, s, *H*-26), 0.89 and 1.51 (1H each, m, *H*-1), 0.93 and 0.95 (3H each, s, *H*-23 and *H*-27), 1.09 and 1.84 (1H each, m, *H*-11), 1.21 and 1.59 (1H each, m, *H*-12), 1.16 and 1.45 (1H each, m, *H*-15), 1.24 and 1.50 (1H each, m, *H*-6), 1.26 and 1.38 (1H each, m, *H*-7), 1.27 and 1.64 (1H each, m, *H*-21), 1.20 (1H, m, *H*-9), 1.47 and 1.86 (1H each, m, *H*-22), 1.46 and 2.19 (1H each, m, *H*-16), 1.49 (1H, m, *H*-18), 1.54 (2H, m, *H*-2), 1.69 (3H, s, *H*-30), 2.29 (1H, m, *H*-13), 3.01 (1H, m, *H*-19), 3.18 (1H, m, *H*-3), 4.16 and 4.58 (1H each, s, *H*-29), 5.35 and 5.44 (1H each, d, J = 12.0 Hz, −OC*H*$_2$−), 6.97 (1H, d, J = 8.0 Hz, Ar−*H*), 7.05 (1H, d, J = 8.0 Hz, Ar−*H*), 7.28 (1H, d, J = 8.0 Hz, Ar−*H*), 7.50–7.57 (2H, m, Ar−*H*), 8.05 (1H, s, N−C*H*), 8.45 (1H, d, J = 8.0 Hz, Ar−*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.28, 152.67, 150.36, 143.07, 133.05, 129.75, 128.23, 127.03, 125.73, 125.08, 124.10, 123.76, 113.75, 109.74, 109.64, 79.16, 60.53, 57.03, 56.65, 55.27, 50.46, 49.47, 46.99, 42.34, 40.66, 38.82, 38.66, 38.33, 37.12, 36.94, 34.17, 32.01, 30.58, 29.64, 27.98, 27.29, 25.47, 21.08, 20.83,

19.36, 18.22, 16.04, 15.92, 15.37, 14.66. IR γ_{max} (neat): 3544, 3469, 3413, 2927, 2869, 1711, 1638, 1617, 1444, 1378, 1280, 1208, 1181, 1152, 1080, 1071, 1039, 979, 883, 782, 617 cm^{-1}. MS (%) for $C_{43}H_{57}N_3O_4$ at *m/z* 678.9357 $[M - H]^+$, 679 (3.1), 660 (18.7), 623 (20.0), 633 (20.3), 599 (32.8), 539 (40.6), 491 (54.6), 364 (100), 314 (15.6), 279 (16.4), 232 (7.0), 202 (26.5). HRMS *m/z* calcd. for $C_{43}H_{57}N_3O_4$ $[M + H]^+$ 680.4422, found 680.4384.

*4.1.5.12. Synthesis of 3{1N(4-benzoylphenyl)-1H-1,2,3-triazol-4yl} methyloxy betulinic acid (**14**).* The title compound prepared by the reaction of propargylated betulinic acid (100 mg, 0.20 mmol) and 1-azido-4benzoyl benzene (45 mg, 0.3 mmol) as per the method described in Section 4.1.5 to furnish **14** (133 mg, 93% yield), mp 261–263 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.62 (3H, s, *H*-25), 0.64 (1H, m, *H*-5), 0.71 (3H, s, *H*-24), 0.72 (3H, s, *H*-26), 0.88 and 1.51 (1H each, m, *H*-1), 0.93 and 0.94 (3H each, s, *H*-23 and *H*-27), 1.10 and 1.85 (1H each, m, *H*-11), 1.22 and 1.58 (1H each, m, *H*-12), 1.17 and 1.46 (1H each, m, *H*-15), 1.23 and 1.51 (1H each, m, *H*-6), 1.25 and 1.39 (1H each, m, *H*-7), 1.28 and 1.65 (1H each, m, *H*-21), 1.21 (1H, m, *H*-9), 1.46 and 1.87 (1H each, m, *H*-22), 1.47 and 2.21 (1H each, m, *H*-16), 1.50 (1H, m, *H*-18), 1.55 (2H, m, *H*-2), 1.68 (3H, s, *H*-30), 2.28 (1H, m, *H*-13), 3.02 (1H, m, *H*-19), 3.17 (1H, m, *H*-3), 4.61 and 4.74 (1H each, s, *H*-29), 5.27 and 5.38 (1H each, d, J = 12.0 Hz, $-OCH_2-$), 7.50–7.54 (2H, m, Ar–*H*), 7.62–7.64 (1H, m, Ar–*H*), 7.82 (2H, d, J = 8.0 Hz, Ar–*H*), 7.90 (2H, d, J = 8.15 Hz, Ar–*H*), 7.99 (2H, d, J = 8.15 Hz, Ar–*H*), 8.19 (1H, s, N–C*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 195.21, 176.27, 150.37, 144.34, 139.45, 137.63, 136.98, 132.98, 131.79 (2× CH), 130.02 (2× CH), 128.56 (2× CH), 122.44, 119.71 (2× CH), 109.78, 78.92, 56.91, 56.57, 55.24, 50.42, 49.49, 47.02, 42.32, 40.61, 38.82, 38.65, 38.41, 37.11, 36.87, 34.14, 31.97, 30.58, 29.60, 27.95, 27.32, 22.73, 20.83, 19.37, 18.20, 16.05, 15.61, 15.40, 14.69. IR γ_{max} (neat): 3446, 3272, 2929, 2869, 1712, 1631, 1618, 1464, 1375, 1247, 1195, 1135, 1071, 1044, 945, 885, 809, 618 cm^{-1}. MS (%) for $C_{46}H_{59}N_3O_4$ at *m/z* 741.1351 $[M + Na]^+$, 717 (18.3), 625 (16.9), 638 (11.2), 546 (21), 563 (71.8), 566 (46.6), 593 (19.7), 493 (21.1), 448 (13.3), 324 (45.0), 263 (71.8), 241.5 (100), 198 (16.9), 172 (25.3), 137 (9.8). HRMS *m/z* calcd. for $C_{46}H_{59}N_3O_4$ $[M + H]^+$ 718.4578, found 718.4531.

*4.1.5.13. Synthesis of 3{1N(2,3,4,6-tetraacetylgalactoso-1yl)-1H-1,2,3-triazol-4yl}methyloxy betulinic acid (**15**).* The title compound prepared by the reaction of propargylated betulinic acid (125 mg, 0.25 mmol) and 1-azido-2,3,4,6-tetra acetyl galactose (114 mg, 0.3 mmol) as per the method described in Section 4.1.5 to furnish **15** (212 mg, ~98% yield), mp 215–217 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.64 (1H, m, *H*-5), 0.72 (3H, s, *H*-25), 0.76 (3H, s, *H*-24), 0.77 (3H, s, *H*-26), 0.88 and 1.51 (1H each, m, *H*-1), 0.93 and 0.94 (3H each, s, *H*-23 and *H*-27), 1.09 and 1.84 (1H each, m, *H*-11), 1.21 and 1.59 (1H each, m, *H*-12), 1.16 and 1.45 (1H each, m, *H*-15), 1.24 and 1.50 (1H each, m, *H*-6), 1.26 and 1.38 (1H each, m, *H*-7), 1.27 and 1.64 (1H each, m, *H*-21), 1.20 (1H, m, *H*-9), 1.47 and 1.86 (1H each, m, *H*-22), 1.46 and 2.18 (1H each, m, *H*-16), 1.49 (1H, m, *H*-18), 1.54 (2H, m, *H*-2), 1.69 (3H, s, *H*-30), 1.91, 2.03, 2.05, 2.25 (3H each, s, 4× $-OCOCH_3$), 2.27 (1H, m, *H*-13), 3.02 (1H, m, *H*-19), 3.18 (1H, m, *H*-3), 4.12–4.15 (1H, m, sugar–*H*), 4.18–4.25 (2H, m, sugar–*H*), 4.60 and 4.75 (1H each, s, *H*-29), 5.22–5.26 (3H, m, $-OCH_2-$ and sugar–*H*), 5.51 (1H, d, J = 8.0 Hz, sugar–*H*), 5.55 (1H, d, J = 2.0 Hz, sugar–*H*), 5.83 (1H, d, J = 9.24 Hz, sugar–*H*), 7.92 (1H, s, N–C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.93, 170.23, 169.99, 169.93, 168.90, 150.42, 143.86, 122.31, 109.72, 86.33, 78.93, 74.01, 70.72, 67.90, 66.73, 60.95, 56.81, 56.54, 55.31, 50.51, 49.39, 46.92, 42.37, 40.65, 38.83, 38.70, 38.21, 37.16, 36.85, 34.25, 31.92, 30.46, 29.59, 27.95, 27.35, 25.48, 20.87, 20.68, 20.61, 20.50, 20.21, 19.27, 18.27, 16.15, 15.76, 15.35, 14.68. IR γ_{max} (neat): 3555, 3369, 2927, 2869, 1711, 1633, 1444, 1378, 1280, 1208, 1181, 1152, 1071, 979, 883, 806, 782, 617 cm^{-1}. MS (%) for $C_{47}H_{69}N_3O_{12}$ at *m/z* 868.4912 $[M + H]^+$, 868 (100), 825 (1.8), 783 (2.6), 741 (25), 699 (9.6), 642 (5.2), 635 (1.8), 483 (4.2), 480 (3.6), 396 (2.4), 394 (3.9), 365 (6.4), 319 (6.9), 271 (7.5), 212 (3.3), 186 (4.1), 169 (4.1), 162 (3.1), 130 (5.1). HRMS *m/z* calcd. for $C_{47}H_{69}N_3O_{12}$ $[M + H]^+$ 868.4954, found 868.4895.

*4.1.5.14. Synthesis of 3{1N(3,4-methylenedioxyphenyl)-1H-1,2,3-triazol-4yl}methyloxy betulinic acid (**16**).* The title compound prepared by the reaction of propargylated betulinic acid (175 mg, 0.35 mmol) and 1-azido-3,4-methylenedioxy benzene (70 mg, 0.43 mmol) as per the method described in Section 4.1.5 to furnish **16** (221 mg, 97% yield), mp 227–229 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.64 (1H, m, *H*-5), 0.65 (3H, s, *H*-25), 0.73 (3H, s, *H*-24), 0.73 (3H, s, *H*-26), 0.89 and 1.51 (1H each, m, *H*-1), 0.93 and 0.94 (3H each, s, *H*-23 and *H*-27), 1.09 and 1.84 (1H each, m, *H*-11), 1.21 and 1.59 (1H each, m, *H*-12), 1.16 and 1.45 (1H each, m, *H*-15), 1.24 and 1.50 (1H each, m, *H*-6), 1.26 and 1.38 (1H each, m, *H*-7), 1.27 and 1.64 (1H each, m, *H*-21), 1.20 (1H, m, *H*-9), 1.47 and 1.86 (1H each, m, *H*-22), 1.46 and 2.05 (1H each, m, *H*-16), 1.49 (1H, m, *H*-18), 1.54 (2H, m, *H*-2), 1.69 (3H, s, *H*-30), 2.29 (1H, m, *H*-13), 3.01 (1H, m, *H*-19), 3.18 (1H, m, *H*-3), 4.60 and 4.73 (1H each, s, *H*-29), 5.23 and 5.34 (1H each, d, J = 12.0 Hz, $-OCH_2-$), 6.91 (1H, d, J = 8.0 Hz, Ar–*H*), 7.13 (1H, dd, J = 2.0, 8.0 Hz, Ar–*H*), 7.24 (1H, d, J = 2.0 Hz, Ar–*H*), 7.97 (1H, s, N–C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.20, 150.41, 148.66, 148.12, 143.72, 131.40, 122.74, 114.26, 109.69, 108.48, 102.85, 102.16, 78.94, 57.04, 56.56, 55.28, 50.47, 49.50, 46.99, 42.32, 40.64, 38.83, 38.68, 38.37, 37.14, 36.86, 34.18, 32.81, 30.59, 29.70, 27.96, 27.36, 25.48, 20.85, 19.37, 18.21, 16.02, 15.72, 15.35, 14.67. IR γ_{max} (neat): 3415, 2942, 2869, 1711, 1639, 1616, 1508, 1465, 1376, 1245, 1181, 1106, 1077, 1038, 934, 884, 807, 612 cm^{-1}. MS (%) for $C_{40}H_{55}N_3O_5$ at *m/z* 658.2445 $[M + H]^+$, 658 (12), 630 (4), 617 (7.5), 589 (11.2), 535 (13.6), 456 (9.4), 428 (17.3), 426 (21.1), 414 (23.1), 385 (100), 360 (27.3), 300 (33.5), 282 (16.3), 121 (47.2), 92 (13.9). HRMS *m/z* calcd. for $C_{40}H_{55}N_3O_5$ $[M + H]^+$ 658.4214, found 658.4176.

*4.1.5.15. Synthesis of 3{1N(4-chlorophenyl)-1H-1,2,3-triazol-4yl}methyloxy betulinic acid (**17**).* The title compound prepared by the reaction of propargylated betulinic acid (100 mg, 0.20 mmol) and 4-azido-chlorobenzene (40 mg, 0.26 mmol) as per the method described in Section 4.1.5 to furnish **17** (122 mg, 94% yield), mp 245–246 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.62 (3H, s, *H*-25), 0.63 (1H, m, *H*-5), 0.72 (3H, s, *H*-24), 0.73 (3H, s, *H*-26), 0.88 and 1.50 (1H each, m, *H*-1), 0.92 and 0.94 (3H each, s, *H*-23 and *H*-27), 1.09 and 1.85 (1H each, m, *H*-11), 1.22 and 1.61 (1H each, m, *H*-12), 1.17 and 1.46 (1H each, m, *H*-15), 1.25 and 1.51 (1H each, m, *H*-6), 1.27 and 1.39 (1H each, m, *H*-7), 1.28 and 1.65 (1H each, m, *H*-21), 1.21 (1H, m, *H*-9), 1.46 and 1.87 (1H each, m, *H*-22), 1.47 and 2.10 (1H each, m, *H*-16), 1.49 (1H, m, *H*-18), 1.55 (2H, m, *H*-2), 1.69 (3H, s, *H*-30), 2.29 (1H, m, *H*-13), 3.01 (1H, m, *H*-19), 3.18 (1H, m, *H*-3), 4.60 and 4.73 (1H each, s, *H*-29), 5.26 and 5.35 (1H each, d, J = 12.0 Hz, $-OCH_2-$), 7.51 (2H, d, J = 8.0 Hz, Ar–*H*), 7.69 (2H, d, J = 8.0 Hz, Ar–*H*), 8.06 (1H, s, N–C*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.23, 150.36, 144.17, 135.40, 134.75, 129.95 (2× CH), 122.35, 121.71 (2× CH), 109.72, 80.99, 56.95, 56.57, 55.29, 50.46, 49.51, 47.00, 42.34, 40.64, 38.83, 38.69, 38.40, 37.14, 36.86, 34.19, 31.98, 30.60, 29.69, 27.95, 27.36, 25.49, 20.85, 19.36, 18.21, 16.01, 15.66, 15.34, 14.67. IR γ_{max} (neat): 3470, 2943, 2868, 1716, 1617, 1509, 1466, 1375, 1246, 1183, 1109, 1081, 1039, 935, 889, 811, 617 cm^{-1}. MS (%) for $C_{39}H_{54}ClN_3O_3$ at *m/z* 647.3895 $[M^+, {}^{35}Cl]$, 649 $[M^+, {}^{35}Cl]$, 649 (12.6), 647 (33.3), 622 (5.5), 620 (16.1), 611 (19.0), 609 (8.7), 578 (44.4), 580 (15.8), 536 (50.7), 508 (60.3), 483 (37.3), 458 (33.3), 423 (47.6), 344 (47.6), 319 (34.9), 295 (7.1), 222 (47.6). HRMS *m/z* calcd. for $C_{39}H_{54}ClN_3O_3$ $[M + H]^+$ 648.3926, found 648.3888.

*4.1.5.16. Synthesis of 3{1N(2,3,4,6-tetraacetylglucoso-1yl)-1H-1,2,3-triazol-4yl}methyloxy betulinic acid (**18**).* The title compound

prepared by the reaction of propargylated betulinic acid (110 mg, 0.22 mmol) and 1-azido-2,3,4,6-tetra acetyl glucose (100 mg, 0.27 mmol) as per the method described in Section 4.1.5 to furnish **3** (185 mg, 97% yield), mp 163–165 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.65 (1H, m, *H*-5), 0.68 (3H, s, *H*-25), 0.74 (3H, s, *H*-24), 0.78 (3H, s, *H*-26), 0.88 and 1.51 (1H each, m, *H*-1), 0.89 and 0.90 (3H each, s, *H*-23 and *H*-27), 1.09 and 1.84 (1H each, m, *H*-11), 1.21 and 1.59 (1H each, m, *H*-12), 1.16 and 1.45 (1H each, m, *H*-15), 1.24 and 1.50 (1H each, m, *H*-6), 1.26 and 1.38 (1H each, m, *H*-7), 1.27 and 1.64 (1H each, m, *H*-21), 1.20 (1H, m, *H*-9), 1.47 and 1.86 (1H each, m, *H*-22), 1.46 and 2.18 (1H each, m, *H*-16), 1.49 (1H, m, *H*-18), 1.54 (2H, m, *H*-2), 1.64 (3H, s, *H*-30), 1.78, 1.94, 1.99, 2.01 (3H each, s, 4× $-OCOCH_3$), 2.29 (1H, m, *H*-13), 3.01 (1H, m, *H*-19), 3.17 (1H, m, *H*-3), 4.12 (1H, m, sugar–*H*), 4.18 (1H, m, sugar–*H*), 4.28 (1H, m, sugar–*H*), 4.53 and 4.64 (1H each, s, *H*-29), 5.11 and 5.18 (1H each, d, $J = 12.76$ Hz, $-OCH_2-$), 5.19–5.22 (1H, m, sugar–*H*), 5.47–5.52 (2H, m, 2× sugar–*H*), 6.10 (1H, d, $J = 8.95$ Hz, sugar–*H*), 8.22 (1H, s, N–C*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 175.84, 170.33, 169.88, 169.31, 168.73, 150.41, 144.01, 122.04, 109.72, 85.80, 78.94, 75.22, 72.57, 70.35, 67.68, 61.68, 56.87, 56.54, 55.31, 50.52, 49.42, 46.89, 42.38, 40.67, 38.84, 38.71, 38.24, 37.16, 36.84, 34.27, 31.92, 30.49, 29.61, 27.97, 27.36, 25.50, 20.85, 20.69, 20.51, 20.49, 20.14, 19.30, 18.27, 16.12, 15.80, 15.35, 14.68. IR γ_{max} (neat): 3437, 2942, 2870, 1756, 1641, 1453, 1375, 1228, 1151, 1104, 1041, 924, 887, 756 cm^{-1}. MS (%) for $C_{47}H_{69}N_3O_{12}$ at *m*/*z* 868.4954 $[M + H]^+$, 868 (100), 642 (1.9), 635 (1.3), 483 (3.7), 480 (2.6), 396 (1.9), 394 (3.5), 365 (2.4), 319 (6.4), 271 (1.5), 212 (0.7), 186 (1.2), 169 (1.8), 162 (1.4), 130 (3.4). HRMS *m*/*z* calcd. for $C_{47}H_{69}N_3O_{12}$ $[M + H]^+$ 868.4954, found 868.49543.

*4.1.5.17. Synthesis of 3{1N(4-nitrophenyl)-1H-1,2,3-triazol-4yl}methyloxy betulinic acid (**19**).* The title compound prepared by the reaction of propargylated betulinic acid (125 mg, 0.25 mmol) and 1-azido-4-nitro benzene (50 mg, 0.3 mmol) as per the method described in Section 4.1.5 to furnish **19** (157 mg, 95% yield), mp 250–253 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.62 (3H, s, *H*-25), 0.63 (1H, m, *H*-5), 0.70 (3H, s, *H*-24), 0.72 (3H, s, *H*-26), 0.87 and 1.51 (1H each, m, *H*-1), 0.93 and 0.94 (3H each, s, *H*-23 and *H*-27), 1.09 and 1.84 (1H each, m, *H*-11), 1.21 and 1.59 (1H each, m, *H*-12), 1.16 and 1.45 (1H each, m, *H*-15), 1.24 and 1.50 (1H each, m, *H*-6), 1.26 and 1.38 (1H each, m, *H*-7), 1.27 and 1.64 (1H each, m, *H*-21), 1.20 (1H, m, *H*-9), 1.47 and 1.86 (1H each, m, *H*-22), 1.46 and 2.19 (1H each, m, *H*-16), 1.49 (1H, m, *H*-18), 1.68 (3H, s, *H*-30), 1.54 (2H, m, *H*-2), 2.29 (1H, m, *H*-13), 3.01 (1H, m, *H*-19), 3.15 (1H, m, *H*-3), 4.61 and 4.74 (1H each, s, *H*-29), 5.28 and 5.35 (1H each, d, $J = 12.0$ Hz, $-OCH_2-$), 7.98 (2H, d, $J = 8.0$ Hz, Ar–*H*), 8.21 (1H, s, N–C*H*), 8.44 (2H, d, $J = 8.0$ Hz, Ar–*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.31, 150.30, 147.33, 144.80, 141.01, 125.59 (2× CH), 122.42, 120.55 (2× CH), 109.83, 78.95, 56.79, 56.59, 55.23, 50.40, 49.45, 47.01, 42.34, 40.62, 38.82, 38.65, 38.42, 37.11, 36.87, 34.15, 31.95, 30.55, 29.61, 27.94, 27.32, 25.48, 20.82, 19.34, 18.20, 16.04, 15.59, 15.37, 14.70. IR γ_{max} (neat): 3468, 2929, 2868, 1712, 1616, 1566, 1445, 1367, 1281, 1209, 1183, 1153, 1081, 1073, 1043, 981, 881, 783, 618 cm^{-1}. MS (%) for $C_{39}H_{54}N_4O_5$ at *m*/*z* 659.4195 $[M + H]^+$, 659 (100), 631 (6), 569 (10), 543 (10), 525 (11), 399 (20.3), 365 (12.5), 321 (20.3), 233 (18.7), 185 (25), 174 (20), 156 (26.5), 135 (14), 102 (12.5). HRMS *m*/*z* calcd. for $C_{39}H_{54}N_4O_5$ $[M + H]^+$ 659.4167, found 659.4111.

*4.1.5.18. Synthesis of 3{1N(3-iodophenyl)-1H-1,2,3-triazol-4yl}methyloxy betulinic acid (**20**).* The title compound prepared by the reaction of propargylated betulinic acid (100 mg, 0.20 mmol) and 1-azido-3-iodo benzene (62 mg, 0.25 mmol) as per the method described in Section 4.1.5 to furnish **20** (136 mg, 92% yield), mp 236–237 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.64 (1H, m, *H*-5), 0.68 (3H, s, *H*-25), 0.73 (3H, s, *H*-24), 0.78 (3H, s, *H*-26), 0.88 and 1.51 (1H each, m, *H*-1), 0.93 and 0.94 (3H each, s, *H*-23 and *H*-27), 1.09 and 1.86 (1H each, m, *H*-11), 1.21 and 1.59 (1H each, m, *H*-12), 1.16 and 1.45 (1H each, m, *H*-15), 1.24 and 1.50 (1H each, m, *H*-6), 1.26 and 1.38 (1H each, m, *H*-7), 1.27 and 1.64 (1H each, m, *H*-21), 1.20 (1H, m, *H*-9), 1.47 and 1.86 (1H each, m, *H*-22), 1.46 and 2.09 (1H each, m, *H*-16), 1.49 (1H, m, *H*-18), 1.54 (2H, m, *H*-2), 1.69 (3H, s, *H*-30), 2.29 (1H, m, *H*-13), 3.02 (1H, m, *H*-19), 3.19 (1H, m, *H*-3), 4.61 and 4.74 (1H each, s, *H*-29), 5.26 and 5.38 (1H each, d, $J = 12.0$ Hz, $-OCH_2-$), 7.25 (1H, m, Ar–*H*), 7.58 (1H, d, $J = 8.0$ Hz, Ar–*H*), 7.71 (1H, d, $J = 1.82$ Hz, Ar–*H*), 7.78 (1H, dd, $J = 8.0$, 1.82 Hz, Ar–*H*), 7.86 (1H, s, N–C*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.17, 150.34, 143.25, 138.78, 133.24, 135.42, 134.25, 133.01, 125.16, 109.68, 93.18, 78.75, 56.93, 56.54, 55.36, 50.47, 49.45, 46.97, 42.35, 40.74, 38.87, 38.71, 38.34, 37.19, 36.93, 34.25, 31.96, 30.54, 29.61, 28.01, 27.36, 25.51, 20.87, 19.40, 18.34, 16.36, 15.86, 15.46, 14.67. IR γ_{max} (neat): 3444, 2929, 2868, 1699, 1631, 1618, 1464, 1377, 1245, 1191, 1135, 1079, 1038, 943, 885, 811, 618 cm^{-1}. MS (%) for $C_{39}H_{54}IN_3O_3$ at *m*/*z* 738.3271 $[M + H]^+$, 738 (7), 698 (2.5), 662 (28.9), 644 (28.1), 628 (12.5), 605 (26.5), 589 (50), 535 (43.7), 486 (32.8), 462 (79.6), 442 (21.8), 437 (31.8), 410 (39), 369 (28.1), 294 (32.8), 252 (37.2), 220 (35.9), 168 (100). HRMS *m*/*z* calcd. for $C_{39}H_{54}IN_3O_3$ $[M + H]^+$ 740.3282, found 740.3215.

4.2. Biology

RPMI-1640 medium, Rhodamine-123 (Rh-123), Propidium iodide (PI), Proteinase K, Penicillin, Streptomycin, Camptothecin, Fetal bovine serum, Mitomycin, Sodium bicarbonate, Phosphate buffer saline (PBS), Sulpharhodamine (SRB), Trypsin, Paclitaxel, 5-Fluorouracil (5-FU), Doxorubicin, Gentamycin sulfate, electrophoresis reagents, were purchased from Sigma chemical Co. Tris buffer, Bromophenol blue were procured from Himedia. Glacial acetic acid was purchased from Fisher Scientific and Trichloroacetic acid (TCA) from Merck Specialties Private Ltd. All these chemicals used in the present study were of molecular biology grade.

4.2.1. Cell culture, growth conditions and treatment

Human promyelocytic leukemia cell line (HL-60), human breast cancer cell line (MCF-7), human neuroblastoma cancer cell line (SF-295), human acute monocytic leukemia cell line (THP-1), human liver cancer cell lines (HEP-2) and normal kidney cell line of monkey (CV-1) were procured from National Centre for Cell Sciences (NCCS), Pune, India. Human lung carcinoma cell line A-549, and human colon cancer cell line (HCT-15) were obtained from National Cancer Institute, Frederick, USA. Human prostate cancer cell line (PC-3) and human prostate cancer cell line (DU-145), were obtained from National Cancer Institute (NCI), Bethesda, USA. All the cells that were used were grown in RPMI-1640/MEM medium containing 10% FCS, 100 unit Penicillin/100 μg Streptomycin per mL medium. Cells were allowed to grown in CO_2 incubator (Thermo Scientific USA) at 37 °C with 98% humidity and 5% CO_2 gas environment.

4.2.2. Sulforhodamine B assay for % growth inhibition

The sulforhodamine B (SRB) assay was used to screen betulinic acid and its structurally modified derivatives for cytotoxicity. The assay is based on the ability of SRB dye to bind to basic protein 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 number. In the present case, to determine the effect of structurally modified derivatives of betulinic acid on cell number over time, SRB assays were performed as described. Cells were seeded in flat-bottomed

96 welled plates. The cells were allowed to adhere overnight, and then media containing different structural analogs of betulinic acid at different concentrations were added. The plates were assayed for 48 h. The cells were fixed by adding 50 μL of ice-cold 50% TCA to each well for 60 min in case of adherent cells and with 80% TCA in case of HL-60 cells which is a suspension cell line. Suspension cells are then centrifuged at 1500 rpm for 15 min to pallet down the cells. 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 wells plate 10 mM Tris–base, shaken for 5 min and the OD was measured at 570 nm with reference wavelength of 620 nm [21]. The results are given in Table S1 (Fig. S1). Further, the IC_{50} values in the presence of different compounds on the cancer cells of different tissue origin used for screening were determined by plotting the graph between concentration *vs* %age growth inhibition (Table 1).

4.2.3. DNA agarose gel electrophoresis for evaluating DNA fragmentation

HL-60 cells (2×10^6 cells/well/2 mL) were grown in 6 welled plates and treated with indicated concentration of compound **7** and **13** for 24 h. After treatment, 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 Tris EDTA (TE) buffer, mixed in loading buffer and electrophoresed in 1% agarose gel at 80 V for 1.5 h in Tris acetate EDTA (TAE) buffer [22].

4.2.4. Measurement of mitochondrial membrane potential for cellular energy status

Loss in mitochondrial transmembrane potential ($\Delta\Psi_m$) as a result of mitochondrial perturbation was measured using confocal microscopy after staining with Rhodamine-123. HL-60 cells (6×10^4/mL/well) were grown in 6 welled plates and treated with indicated concentration of compound **7** and **13**. Rh-123 (1 μM) was added 30 min before the termination of experiment in dark in the serum free media. After 24 h the cells were washed in PBS and centrifuged at 1500 rpm for 5 min and suspended in PBS. The decrease in intensity of fluorescence because of mitochondrial membrane potential loss was analyzed using confocal microscopy (laser 488) (Olympus) [23].

4.2.5. In vitro clonogenic assay

Clonogenic assay was performed to evaluate the ability of a cell to grow into a colony. The cells were harvested from logarithmically growing stock cultures and plated at the required final clonogenic dilutions prior to treatment. After 24 h the cells were treated with indicated concentrations of the test molecules for another 24 h. After treatment, the plates were placed in an incubator for a time equivalent to at least six potential cell divisions (to give colonies of >50 cells). After this the medium was aspirated from the plates so as not to disturb the colonies. Plates are then washed with PBS and then a mixture of 6% glutaraldehyde and 0.5% crystal violet were added for 30 min to allow sufficient staining. The mixture was removed after 30 min and the plates were washed with water and allowed to dry at room temperature [24,25].

4.2.6. In vitro cell migration assay

The wound-healing assay is simple, inexpensive, and one of the earliest developed methods to study directional cell migration *in vitro*. The basic steps involve creating a "wound" in a cell monolayer, capturing the images at the beginning and at regular intervals during cell migration to close the wound and comparing the images to quantify the migration rate of the cells. 3×10^5 cells/mL/well were seeded in 6 wells plate. Cells were then wounded by scratching with a 1000 μL pipette tip cell was then washed three times with PBS to remove cell debris. The test samples were added at the desired concentration and the plates were incubating in the CO_2 incubator after another 24 h. After the completion of the treatment, the fresh medium was added into the plates and the plates were again incubated till the control wound gets completely healed. The wounds were photographed (10× objective) at 0 h and 24 h and healing was quantified by measuring the distance between the edges of the wound [26].

Acknowledgements

The authors are thankful to Dr. SN Sharma and Mr. S. Kitchlu (Taxonomists, IIIM Jammu) for providing authentic *Platanus orientalis* plant material. Authors (RM, PKC, IK and NAD) thank Council of Scientific and Industrial Research (CSIR-UGC), New Delhi for the award of research fellowships. Staff of instrumentation division (NMR) of our institute is well acknowledged for recording the spectroscopic data.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.03.028.

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