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RESEARCH ARTICLE

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Brevifoliol ester induces apoptosis in prostate cancer cells by activation of caspase pathway

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Abstract

Prostate cancer is fourth most abundant cancer type around the globe. Brevifoliol, a rearranged taxoid from *Taxus walllichiana* needles has been derivatized as C5 esters using Steglich esterification reaction. Seventeen diverse analogues were evaluated against a panel of human cancer cell lines by MTT assay. Among these, two of the semi-synthetic analogues, that is, **13** and **16** exhibited potent cytotoxicity, selectively against PC-3, prostate cancer cell lines. In cell cycle analysis, analogue **13** induced S and G2/M phase arrest and induced apoptosis by activating caspase-3. Compound **13** showed moderate efficacy in in-vivo Ehrlich ascites carcinoma in Swiss albino mice. Further, compound **13** was found to be safe in Swiss albino mice up to 1,000 mg/kg dose in acute oral toxicity. Brevifoliol ester **13** may further be optimized for better efficacy.

KEYWORDS

acute oral toxicity, anticancer, apoptosis, brevifoliol analogues, Ehrlich ascites carcinoma

1 | INTRODUCTION

Cancer is one of the leading causes of morbidity and mortality worldwide. It is second leading cause of deaths globally and estimated responsible for 9.6 million deaths in 2018 (WHO factsheet, 2018). The economic impact of cancer is significantly increasing which was approximately US$ 1.16 trillion in 2010. Cancer can be prevented by increasing fruit and vegetable intakes and avoiding tobacco and alcohol. Many cancers have a high chance of cure if diagnosed early and treated adequately. Nevertheless, there are no such medicines which can cure completely.

Prostate cancer is fourth most abundant cancer type around the globe. Worldwide, there are ~1.28 million cases of prostate cancer in 2018 (WHO factsheet, 2018). Usually, prostate cancer is initially confined to prostate gland but aggressive and fast spreading type prostate cancer should be treated early. Presently, flutamide, docetaxel, cabazitaxel, abiraterone, enzalutamide, apalutamide, mitoxantrone etc. are first line drugs with a lot of side effects (Yoo, Choi, You, & Kim, 2016. Further advancement may cause metastasis, urinary incontinence, and erectile dysfunction. There is still a need for effective, safe and affordable anti-prostate cancer drug.

Taxus wallichiana (Taxaceae) is grown in the Himachal ranges of Himalaya in India. Paclitaxel is the most important taxoid from this plant which is a clinical drug for various cancers like ovarian, breast, cervical, lung, pancreatic, Kaposi sarcoma. Brevifoliol is a rearranged taxoid having an 11 (15 → 1) abeotaxane system isolated from *Taxus wallichiana*

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(Chattopadhyay, Sharma, Appendino, & Gariboldi, 1995), Needles of Himalayan Yew are relatively rich in brevifoliol as compared to paclitaxel. In this report, we have synthesized seventeen semi-synthetic analogues through Steglich esterification at C-5 position of brevifoliol by using diverse fragments of trimethoxy benzoic acid, trimethoxycinnamic acid, trimethoxy phenyl acetic acid, angelic acid, and other hetero atom on aryl moiety. Two of the semi-synthetic derivatives exhibited potent cytotoxicities against human prostate cancer cells. The best derivative has further been explored for extensive pharmacology (Figure 1).

2 | EXPERIMENTAL

2.1 | Chemistry

2.1.1 | Isolation of brevifoliol (1)

68 Kg needles of *Taxus wallichiana* were collected from Jageswar, Dist Almora, Uttarakhand, India. Plant was authenticated by Pharmacognosist at CSIR-CIMAP, Lucknow (Repository Ref. no. T026). Plant material was shade dried (13.7 kg) and powdered (Sieve no. 16). 13 kg of material was successively extracted with increasing polarity of organic solvents. Firstly, it was treated with hexane (20Ltx4), followed by chloroform (20Ltx5) and ethyl acetate (20Ltx5) to get extracts 206.89 g (Hexane fraction), 66.74 g (Chloroform fraction), and 82.81 g (Ethyl acetate fraction). Ethyl acetate fraction (40 g) was charged on a silica gel column (600 g, 60–120 mesh) and eluted with chloroform, acetone-chloroform (up to 1%–10%), and methanol-chloroform (up to 1%–8%). Pure brevifoliol (0.79 g) was obtained at 6%–8% methanol-chloroform.

*Brevifoliol (**1**):* Yield = 4.2g (0.05%), m.p. = 202–204°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (s, 3H, 19-CH_3), 1.03(s, 3H, 16-CH_3), 1.28 (bs, 2H, 14-CH_2), 1.33(s, 3H, 17-CH_3), 1.41, 2.35 (bs, 2H, 2-CH_2), 1.83 (s, 3H, 18-CH_3), 1.86 (bm, 2H, CH_2), 2.05 (s, 3H, OAc), 2.12 (s, 3H, OAc), 1.49 & 2.46(m, 2H, CH_2), 2.77 (bd, 1H, 3-CH), 4.38 (bs, 2H, 5-CH and 13-CH), 4.36 (s, 1H, 7- CH), 5.16 (s, 1H, 9-CH), 5.56 (bm, 1H, 10-CH), 6.03, 6.52 (bs, 2H, 20-CH_2), 7.42 (m, 2H, CH aromatic), 7.54 (m, 1H, CH aromatic), 7.85 (m, 2H, CH aromatic); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.02, 13.11, 21.32, 21.45, 24.86, 26.91, 29.15, 36.12, 45.08, 47.37, 62.56, 70.26, 70.39, 70.92, 72.55, 75.31, 75.94, 76.09, 112.04, 128.75, 129.37, 129.44, 133.26, 134.03, 149.14, 151.55, 164.38, 169.97, 170.54; ESI-MS for $C_{31}H_{40}O_9$ (MeOH): 557 $[M + H]^+$.

2.1.2 | General procedure for the synthesis of C5 esters of brevifoliol (2–18)

*Synthesis of Brevifoliol-5-O-yl-angelic acid ester (**2**)*

Angelic acid (23 mg, 0.23 mmol), dicyclohexylcarbodiimide (18.5 mg, 0.188 mmol), and 4-dimethylaminopyridine (11 mg, 0.09 mmol) were stirred in dry dichloromethane

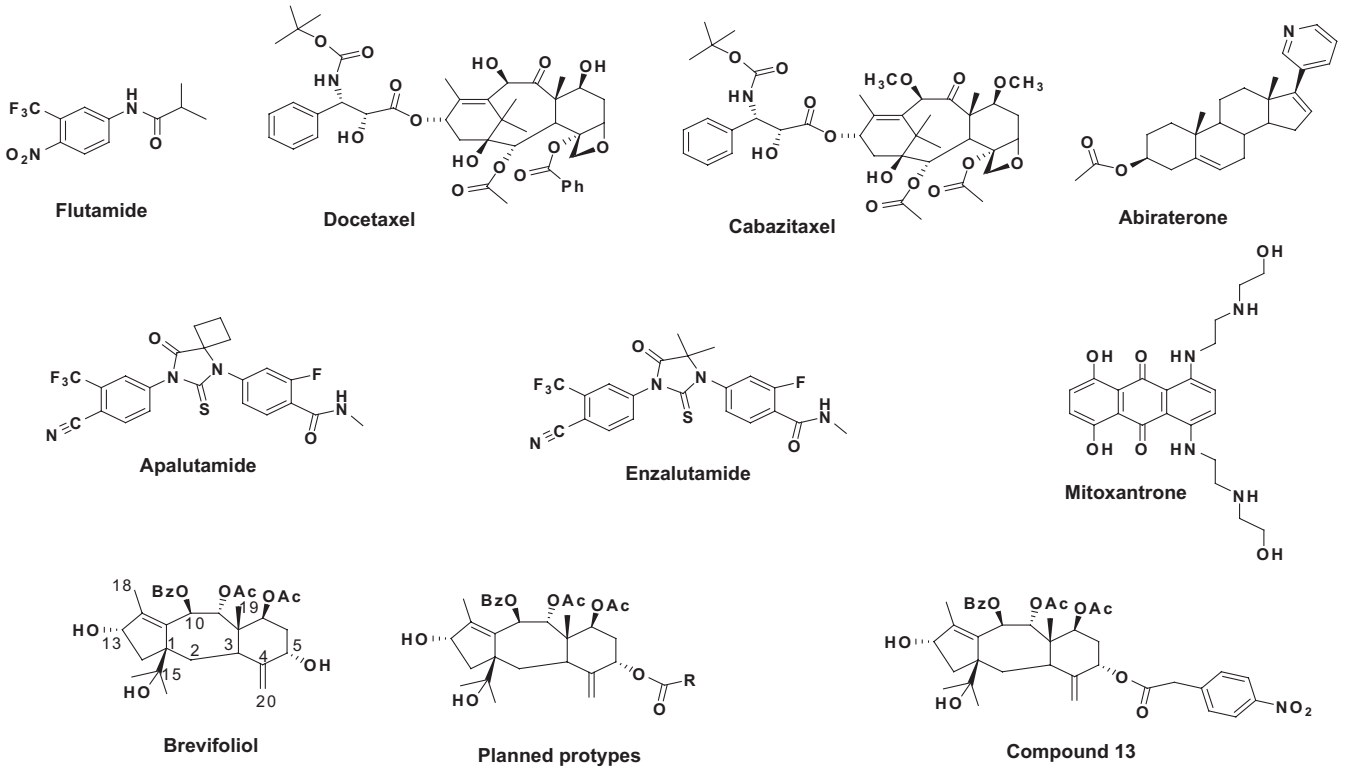


FIGURE 1 Structures of clinical drugs for prostate cancer, brevifoliol, planned prototype, and derivative **13**

(5 ml) at RT for 30 min. Brevifoliol (50 mg, 0.09 mmol) in dichloromethane (5 ml) was added, and reaction mixture was further stirred for 3 hr. The reaction mixture was diluted with dichloromethane, washed with water, and organic layer dried with anhyd. Na_2SO_4 and concentrated *in-vacuo*. The residue was purified by column chromatography to get gummy compound **7** at 8% acetone/chloroform.

2. Yield = 84%; Yellow gum; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.90 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 1.28 (bs, 2H, CH_2), 1.34 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 1.86 (bm, 2H, CH_2), 2.02 (s, 3H, CH_3), 2.06 (s, 6H, 2xOAc), 1.49, 2.48 (m, 2H, CH_2), 2.37 (bt, 1H, CH), 2.76 (bd, 1H, CH), 4.47 (bs, 1H, CH), 4.88 (s, 1H, CH), 5.08 (s, 1H, CH_2), 5.40 (s, 1H, CH_2), 5.43 (d, 3H, CH_3), 5.59 (bm, 1H, CH), 6.06 (bs, 1H, CH), 6.50 (d, 1H, CH), 6.87 (q, 1H, CH) 7.32 (m, 2H, CH aromatic), 7.52 (m, 1H, CH aromatic), 7.85 (m, 2H, CH aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 12.17, 14.09, 14.47, 20.34, 20.73, 21.40, 24.70, 25.23, 32.71, 39.17, 49.31, 59.80, 69.80, 70.40, 70.39, 71.01, 73.97, 75.75, 76.83, 114.06, 128.30, 128.72, 129.45, 133.24, 133.28, 138.22, 147.55, 153.66, 154.85, 164.18, 166.56, 169.87, 169.92, 175.10. Electrospray mass for $C_{36}H_{46}O_{10}$ (MeOH): 661 $[M + Na]^+$.

*Brevifoliol-5-O-yl-(4'ethyl) benzoic acid ester (**3**).* Yield = 66%; Yellowish gum; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 1.27 (bs, 2H, CH_2), 1.33 (s, 3H, CH_3), 1.76 (bm, 2H, CH_2), 2.00 (s, 3H, CH_3), 2.06 (s, 6H, 2xOAc), 1.49, 2.45 (m, 2H, CH_2), 2.34 (bt, 1H, CH), 2.77 (bd, 1H, CH), 3.00 (s, 2H, CH_2), 1.32 (s, 3H CH_3), 4.39 (bs, 1H, 13-CH), 4.95 (s, 1H, CH_2), 5.35 (s, 1H, CH_2), 5.62 (bm, 1H, CH), 6.10 (bs, 1H, CH), 6.50 (d, 1H, CH), 7.43 (m, 2H, CH aromatic), 7.53 (m, 1H, CH aromatic), 7.85 (m, 2H, CH aromatic), 7.96 (d, 2H, CH aromatic), 8.02 (d, 2H, CH aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.73, 13.00, 15.27, 20.76, 21.40, 24.46, 24.94, 29.70, 32.26, 33.93, 39.11, 44.91, 47.84, 57.25, 69.77, 70.38, 74.52, 75.83, 76.79, 114.53, 126.92, 128.02, 128.76, 129.48, 129.48, 129.90, 133.28, 134.41, 147.54, 148.45, 154.64, 164.21, 165.26, 169.88, 170.03, 171.50. Electrospray mass for $C_{40}H_{48}O_{10}$ (MeOH): 711 $[M + Na]^+$.

*Brevifoliol-5-O-yl-4′-methoxybenzoic acid ester (**4**).* Yield = 53%; Yellowish gum; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.87 (s, 3H, CH_3), 1.10 (s, 3H, CH_3), 1.26 (bs, 2H, CH_2), 1.34 (s, 3H, CH_3), 1.97 (bm, 2H, CH_2), 2.03 (s, 3H, CH_3), 2.07 (s, 6H, 2xOAc), 1.51, 1.78 (m, 2H, CH_2), 2.36 (bt, 1H, CH), 2.78 (bd, 1H, CH), 3.81 (s, 3H, OCH_3), 4.93 (s, 1H, CH_2), 5.32 (s, 1H, CH_2), 5.62 (bm, 1H, CH), 6.10 (bs, 1H, CH), 6.46 (d, 1H, CH), 6.91 (dd, 2H, CH aromatic, J = 6.5 Hz, 7 Hz), 7.42 (m, 2H, CH aromatic), 7.53 (m, 1H, CH aromatic), 7.86 (m, 2H, CH aromatic), 8.00 (d, 2H, CH aromatic, 9 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.77, 12.99, 20.75, 21.39, 24.51, 24.94, 26.24, 27.13, 29.67, 30.82, 32.33, 33.91, 39.05, 49.63, 55.43, 57.26, 69.80, 70.44, 74.41, 75.84, 76.70, 106.58, 113.72, 114.36, 122.40, 128.32, 128.75, 129.09, 129.46, 131.93, 133.27, 148.75, 154.52, 154.78, 161.79, 163.64, 164.22, 165.26, 169.90, 170.02, 170.94; Electrospray mass for $C_{41}H_{48}O_{11}$ (MeOH): 713 $[(M\text{-}CH_2CO)+K]^+$.

*Brevifoliol-5-O-yl-(3′,4′,5-trimethoxyphenyl)-prop-2′-enoic acid ester (**5**).* Yield = 83%; Yellowish gum; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 1.28 (bs, 2H, CH_2), 1.34 (s, 3H, CH_3), 1.96 (bm, 2H, CH_2), 2.03 (s, 3H, CH_3), 2.06 (s, 6H, 2xOAc), 1.74, 2.40 (m, 2H, CH_2), 2.34 (bt, 1H, CH), 2.71 (bd, 1H, CH), 3.87 (s, 6H, 2xOCH_3), 3.93 (s, 3H, OCH_3) 4.76 (bs, 1H, CH), 5.02 (s, 1H, CH), 5.44 (s, 1H, CH_2), 5.54 (s, 1H, CH), 5.55 (bm, 1H, CH), 6.03 (bs, 1H, CH), 6.80 (d, 1H, CH), 7.32 (s, 2H, aromatic), 7.42 (m, 2H, CH aromatic), 7.53 (m, 1H, CH aromatic), 7.85 (m, 2H, CH aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.11, 20.75, 21.40, 22.70, 24.92, 29.70, 31.92, 33.90, 44.97, 49.22, 56.34, 60.95, 69.75, 70.13, 70.36, 75.30, 75.82, 106.80, 114.07, 118.90, 124.91, 128.77, 129.46, 129.72, 133.26, 133.33, 147.81, 150.56, 150.88, 153.06, 153.24, 164.30, 164.80, 165.94, 169.93, 170.03, 170.15. Electrospray mass for $C_{41}H_{50}O_{13}$ (MeOH): 773 $[M + Na]^+$.

*Brevifoliol-5-O-yl-(2′,4′-methylendioxyphenyl)-prop-2′-enoic acid ester (**6**).* Yield = 43%; Yellowish gum; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (s, 3H, CH_3), 1.23 (s, 3H, CH_3), 1.28 (bs, 2H, CH_2), 1.33 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 2.06 (s, 6H, 2xOAc), 1.57, 2.45 (m, 2H, CH_2), 2.34 (bt, 1H, CH), 2.36 (bd, 1H, CH), 4.32 (bs, 1H, CH), 4.81 (s, 1H, CH_2), 4.92 (s, 1H, CH), 5.00 (bm, 1H, CH), 5.80 (s, 1H, CH_2), 6.00 (s, 2H, CH_2), 6.07 (bs, 1H, CH), 6.60 (d, 1H, CH), 6.79 (s, 1H, CH aromatic), 7.35 (m, 2H, CH aromatic), 7.51 (s, 1H, aromatic), 7.52 (s, 1H, aromatic), 7.53 (m, 1H, CH aromatic), 7.72 (m, 2H, CH aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 13.70, 14.10, 19.18, 22.68, 24.51, 25.40, 29.16, 37.11, 38.00, 49.62, 57.60, 65.56, 70.26, 70.39, 71.01, 72.55, 75.31, 76.09, 112.04, 128.75, 129.37, 130.76, 130.89, 132.35, 139.30, 147.80, 150.00, 154.54, 162.13, 167.70, 170.63; Electrospray mass for $C_{39}H_{44}O_{12}$ (MeOH): 727 $[M + Na]^+$.

*Brevifoliol-5-O-yl-(3′-chlorophenyl)-benzoic acid ester (**7**).* Yield = 86%; Yellowish gum; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 1.24 (bs, 2H, CH_2), 1.33 (s, 3H, CH_3), 1.76 (bm, 2H, CH_2), 2.05 (s, 3H, CH_3), 2.06 (s, 6H, 2xOAc), 1.49, 2.40 (m, 2H, CH_2), 2.38 (bt, 1H, CH), 2.86 (bd, 1H, CH), 4.46 (bs, 1H, CH), 4.98 (s, 1H, CH), 5.36 (s, 1H, CH_2), 5.66 (s, 1H, CH_2), 6.03 (bs, 1H, CH), 6.48 (d, 1H, CH), 7.42 (m, 2H, CH aromatic), 7.54 (m, 1H, CH aromatic), 7.85 (m, 2H, CH aromatic),

7.87 (d, 1H, CH aromatic), 7.96 (d, 1H, CH aromatic), 8.01 (d, 1H, CH aromatic), 8.17 (d, 1H, CH aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 11.80, 20.76, 21.40, 24.53, 22.39, 24.94, 29.35, 33.94, 39.06, 47.36, 49.77, 69.71, 70.43, 70.39, 71.01, 72.55, 75.74, 76.80, 115.01, 128.33, 128.77, 129.48, 129.48, 133.30, 134.51, 138.57, 145.03, 148.94, 151.30, 164.28, 165.11, 169.92, 169.97, 170.01. Electrospray mass for $C_{38}H_{43}O_{10}Cl$ (MeOH): 717 $[M + Na]^+$.

Brevifoliol-5-O-yl-(3′,5′-dinitrophenyl)-benzoic acid ester (**8**). Yield = 83%; Yellowish gum; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 1.27 (bs, 2H, CH_2), 1.33 (s, 3H, CH_3), 1.72 (bm, 2H, CH_2), 2.05 (s, 3H, CH_3), 2.14 (s, 6H, 2xOAc), 1.49, 2.40 (m, 2H, CH_2), 2.41 (bt, 1H, CH), 2.77 (bd, 1H, CH), 4.37 (bs, 1H, CH), 4.40 (s, 1H, CH), 4.80 (s, 1H, CH_2), 5.15 (s, 1H, CH_2), 5.56 (bm, 1H, CH), 6.03 (bs, 1H, CH), 6.53 (d, 1H, CH), 7.41 (m, 2H, CH aromatic), 7.54 (m, 1H, CH aromatic), 7.85 (m, 2H, CH aromatic), 8.73 (s, 1H, CH aromatic), 9.20 (d, 2H, CH aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 12.90, 14.11, 20.74, 21.46, 22.68, 25.60, 29.18, 36.13, 39.63, 45.09, 47.26, 62.55, 70.45, 72.43, 75.97, 76.80, 114.06, 122.30, 128.76, 129.36, 129.43, 129.50, 133.27, 134.15, 135.35, 149.30, 151.58, 156.16, 164.39, 165.42, 169.80, 169.79, 170.50. Electrospray mass for $C_{36}H_{42}N_2O_{14}$ (MeOH): 770 $[M + H_3O]^+$.

Brevifoliol-5-O-yl-(4′-methoxyphenyl)-acetate (**9**). Yield = 79%; Yellowish gum; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 1.28 (bs, 2H, CH_2), 1.33 (s, 3H, CH_3), 1.85 (bm, 2H, CH_2), 2.00 (s, 3H, CH_3), 2.06 (s, 6H, 2xOAc), 1.49, 2.45 (m, 2H, CH_2), 2.34 (bt, 1H, CH), 2.77 (bd, 1H, CH), 3.71 (s, 2H, CH_2), 3.78 (s, 3H, OCH3), 4.37 (bs, 1H, CH), 4.41 (s, 1H, CH), 4.81 (s, 1H, CH_2), 5.16 (s, 1H, CH_2), 5.55 (bm, 1H, CH), 6.03 (bs, 1H, CH), 6.52 (d, 1H, CH), 7.42 (m, 2H, CH aromatic), 7.53 (m, 1H, CH aromatic), 7.85 (m, 2H, CH aromatic), 7.86 (d, 2H, CH aromatic), 8.18 (d, 2H, CH aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 13.11, 14.96, 20.75, 21.40, 22.39, 24.86, 29.15, 36.12, 38.00, 39.06, 45.08, 47.37, 62.56, 70.26, 70.39, 71.01, 72.55, 75.31, 76.09, 112.04, 114.06, 128.33, 128.75, 129.37, 129.44, 133.24, 133.26, 134.03, 149.14, 151.55, 154.54, 164.38, 169.91, 170.36; Electrospray mass for $C_{36}H_{42}N_2O_{14}$ (MeOH): 770 $[M + H_3O]^+$.

Brevifoliol-5-O-yl-(2′,4′-dimethoxyphenyl)-acetate (**10**). Yield = 81%; Yellowish gum; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 1.28 (bs, 2H, CH_2), 1.33 (s, 3H, CH_3), 1.85 (bm, 2H, CH_2), 2.00 (s, 3H, CH_3), 2.06 (s, 6H, 2xOAc), 1.49, 2.45 (m, 2H, CH_2), 2.34 (bt, 1H, CH), 2.77 (bd, 1H, CH), 3.66 (s, 2H, CH_2), 3.75 (s, 6H, 2xOCH_3) 4.37 (bs, 1H, CH), 4.41 (s, 1H, CH), 4.81 (s, 1H, CH_2), 5.16 (s, 1H, CH_2), 5.55 (bm, 1H, CH), 6.03 (bs, 1H, CH), 6.52 (d, 1H, CH), 7.42 (m, 2H, CH aromatic), 7.53 (m, 1H, CH aromatic), 7.84 (m, 2H, aromatic), 7.85 (m, 2H, CH aromatic), 8.16 (s, 1H, CH aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 13.11, 14.96, 20.75, 21.40, 22.39, 24.86, 29.15, 36.12, 38.00, 45.08, 47.37, 39.01, 62.56, 70.26, 70.39, 71.01, 72.55, 75.31, 76.09, 104.41, 106.57 112.04, 128.72, 128.75, 129.33, 129.37, 129.44, 133.26, 134.03, 149.14, 151.55, 154.29, 154.23, 164.38, 169.91, 170.00, 171.38; Electrospray mass for $C_{41}H_{50}O_{12}$ (MeOH): 757 $[M + Na]^+$.

Brevifoliol-5-O-yl-(3′,4′,5′-trimethoxyphenyl)-acetate (**11**). Yield = 76%; Yellowish gum; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 1.28 (bs, 2H, CH_2), 1.33 (s, 3H, CH_3), 1.85 (bm, 2H, CH_2), 2.00 (s, 3H, CH_3), 2.06 (s, 6H, 2xOAc), 1.49, 2.45 (m, 2H, CH_2), 2.34 (bt, 1H, CH), 2.77 (bd, 1H, CH), 3.65 (s, 2H, CH_2), 3.8 (s, 3H 3xOCH_3), 4.37 (bs, 1H, CH), 4.41 (s, 1H, CH), 4.81 (s, 1H, CH_2), 5.16 (s, 1H, CH_2), 5.55 (bm, 1H, CH), 6.03 (bs, 1H, CH), 6.52 (d, 1H, CH), 7.42 (m, 2H, CH aromatic), 7.53 (m, 1H, CH aromatic), 7.85 (m, 2H, CH aromatic), 8.12 (s, 2H, CH aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 13.11, 14.96, 20.75, 21.40, 22.39, 24.86, 29.15, 36.12, 38.00, 38.97, 45.08, 47.37, 56.05, 62.56, 70.26, 70.39, 71.01, 72.55, 75.31, 76.09, 112.04, 128.75, 129.37, 129.44, 130.43, 133.16, 133.26, 134.03, 149.14, 151.55, 153.16, 164.38, 169.91, 170.54, 171.40; Electrospray mass $C_{42}H_{52}O_{13}$ (MeOH): 787 $[M + Na]^+$.

Brevifoliol-5-O-yl-(4-chlorophenyl)-acetate (**12**). Yield = 87%; Yellowish gum; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 1.28 (bs, 2H, CH_2), 1.33 (s, 3H, CH_3), 1.85 (bm, 2H, CH_2), 2.00 (s, 3H, CH_3), 2.06 (s, 6H, 2xOAc), 1.49, 2.45 (m, 2H, CH_2), 2.34 (bt, 1H, CH), 2.77 (bd, 1H, CH), 3.70 (s, 2H, CH_2), 4.37 (bs, 1H, CH), 4.41 (s, 1H, CH), 4.81 (s, 1H, CH_2), 5.16 (s, 1H, CH_2), 5.55 (bm, 1H, CH), 6.03 (bs, 1H, CH), 6.52 (d, 1H, CH), 7.21 (m, 2H, CH aromatic), 7.25 (m, 2H, CH aromatic), 7.40 (m, 2H, CH aromatic), 7.84 (m, 1H, CH aromatic), 8.16 (m, 2H, CH aromatic); ^{13}C NMR ($CDCl_3$): δ 13.11, 14.96, 20.75, 21.40, 22.39, 24.86, 29.15, 36.12, 38.00, 45.08, 47.10, 47.37, 62.56, 70.26, 70.39, 71.01, 72.55, 75.31, 76.09, 112.04, 128.75, 129.37, 129.44, 129.47, 130.60, 130.94, 133.26, 134.03, 149.14, 151.55, 164.38, 169.91, 170.54, 171.10. Electrospray mass for $C_{39}H_{45}ClO_{10}$ (MeOH): 731 $[M + Na]^+$.

Brevifoliol-5-O-yl-(4′-nitrophenyl)-acetate (**13**). Yield = 82%; Yellowish gum; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 1.28 (bs, 2H, CH_2), 1.32 (s, 3H, CH_3), 1.87 (bm, 2H, CH_2), 2.00 (s, 3H, CH_3), 2.05 (s, 6H, 2xOAc), 1.49 & 2.45 (m, 2H, CH_2), 2.34 (bt, 1H, CH), 2.97 (bd, 1H, CH), 3.82 (s, 2H, CH_2), 4.37 (bs, 1H, CH), 4.36 (s, 1H, CH), 4.76 (s, 1H, CH_2), 5.11 (s, 1H, CH_2), 5.55 (bm, 1H, CH), 6.03 (bs, 1H, CH), 6.52 (d, 1H, CH), 7.41 (m, 2H, CH aromatic), 7.51 (m, 1H, CH aromatic),

7.82 (m, 2H, CH aromatic), 7.84 (m, 2H, CH aromatic), 8.12 (m, 2H, CH aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 11.91, 13.83, 20.67, 21.40, 22.36, 24.84, 29.13, 34.81, 38.94, 47.18, 48.88, 55.66, 70.55, 72.10, 75.84, 76.09, 111.46, 124.10, 128.70, 129.32, 129.36, 130.23, 130.28, 133.14, 146.90, 149.61, 153.70, 154.30, 164.20, 169.97, 170.12; Electrospray mass for $C_{39}H_{45}NO_{12}$ (MeOH): 742 $[M + Na]^+$.

Brevifoliol-5-O-yl-(4′-trifluorophenyl)-acetate (**14**). Yield = 74%; Yellowish gum; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 1.28 (bs, 2H, CH_2), 1.33 (s, 3H, CH_3), 1.85 (bm, 2H, CH_2), 2.00 (s, 3H, CH_3), 2.06 (s, 6H, 2xOAc), 1.49, 2.45 (m, 2H, CH_2), 2.34 (bt, 1H, CH), 2.77 (bd, 1H, CH), 3.76 (s, 2H, CH_2), 4.37 (bs, 1H, CH), 4.41 (s, 1H, CH), 4.81 (s, 1H, CH_2), 5.16 (s, 1H, CH_2), 5.55 (bm, 1H, CH), 6.03 (bs, 1H, CH), 6.52 (d, 1H, CH), 7.42 (m, 2H, CH aromatic), 7.53 (m, 1H, CH aromatic), 7.81 (m, 2H, CH aromatic), 7.83 (m, 1H, CH aromatic), 7.85 (m, 2H, CH aromatic), 8.12 (s, 1H, CH, aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 13.11, 14.96, 20.75, 21.40, 22.39, 24.86, 29.15, 36.12, 38.00, 38.97, 45.08, 47.37, 62.56, 70.26, 70.39, 71.01, 72.55, 75.31, 76.09, 112.04, 124.00, 126.00, 126.34, 128.75, 129.94, 129.37, 129.44, 133.26, 133.29, 134.03, 135.22, 139.84, 149.14, 151.55, 164.38, 169.91, 170.54, 170.82. Electrospray mass for $C_{40}H_{45}F_3O_{10}$ (MeOH): 765 $[M + Na]^+$.

Brevifoliol-5-O-yl-(4′-aminophenyl)-acetate (**15**). Yield = 49%; yellowish gum; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 1.28 (bs, 2H, CH_2), 1.33 (s, 3H, CH_3), 1.85 (bm, 2H, CH_2), 2.00 (s, 3H, CH_3), 2.06 (s, 6H, 2xOAc), 1.49, 2.45 (m, 2H, CH_2), 2.34 (bt, 1H, CH), 2.77 (bd, 1H, CH), 3.6 (bs, 2H, NH_2), 3.91 (s, 2H, CH_2), 4.37 (bs, 1H, CH), 4.41 (s, 1H, CH), 4.81 (s, 1H, CH_2), 5.16 (s, 1H, CH_2), 5.55 (bm, 1H, CH), 6.03 (bs, 1H, CH), 6.52 (d, 1H, CH), 6.61 (m, 2H, CH aromatic), 7.0 (m, 2H, CH aromatic), 7.40 (m, 2H, CH aromatic), 7.85 (m, 1H, CH aromatic), 8.15 (d, 2H, CH aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 13.11, 14.96, 20.75, 21.40, 22.39, 24.86, 29.15, 36.12, 38.00, 45.08, 47.37, 62.56, 70.26, 70.39, 71.01, 72.55, 75.31, 76.09, 112.04, 115.66, 124.24, 128.75, 129.37, 129.44, 130.44, 133.26, 134.03, 149.14, 149.86, 151.55, 164.38, 169.91, 170.54, 172.10; Electrospray mass for $C_{39}H_{47}NO_{10}$ (MeOH): 712 $[M + Na]^+$.

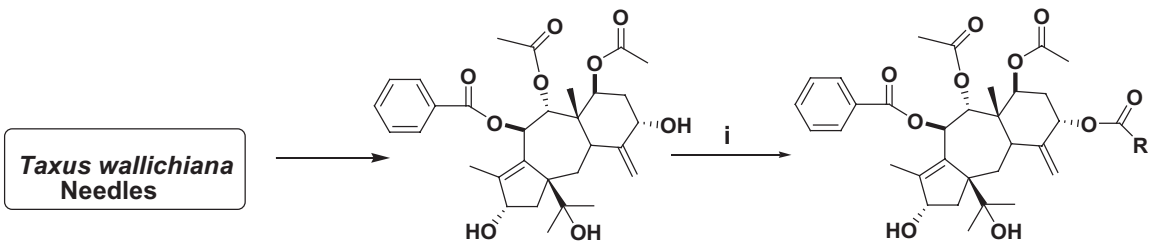
Brevifoliol-5-O-yl-(4′-methoxyphenyl)-prop-2′-enoic acid ester (**16**). Yield = 46%; Yellowish gummy; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 1.28 (bs, 2H, CH_2), 1.33 (s, 3H, CH_3), 1.94 (bm, 2H, CH_2), 2.00 (s, 3H, CH_3), 2.06 (s, 6H, 2xOAc), 1.49, 2.45 (m, 2H, CH_2), 2.34 (bt, 1H, CH), 2.77 (bd, 1H, CH), 3.82 (s, 3H, OCH_3), 4.91 (s, 1H, CH), 5.32 (s, 1H, CH_2), 5.52 (s, 1H, CH_2), 5.55 (bm, 1H, CH), 6.03 (bs, 1H, CH), 6.52 (d, 1H, CH), 6.67 (d, 1H, CH), 6.80 (s, 2H, CH aromatic), 6.88 (d, 2H, CH aromatic), 7.42 (m, 2H, CH aromatic), 7.53 (m, 1H, CH aromatic), 7.57 (d, 1H, CH), 7.87 (m, 2H, CH aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 13.00, 14.11, 20.78, 21.41, 22.70, 24.94, 29.70, 33.94, 39.02, 47.51, 49.08, 55.32, 63.03, 69.83, 70.35, 70.85, 71.01, 72.55, 74.00, 76.80, 114.30, 115.90, 117.00, 127.22, 128.77, 129.48, 129.88, 133.91, 136.27, 139.28, 144.90, 145.71, 154.34, 161.37, 164.23, 166.18, 169.97, 170.04; Electrospray mass for $C_{41}H_{48}O_{11}$ (MeOH): 716.32 $[M + Na]^+$.

Brevifoliol-5-O-yl-(3′,4′,5′-trimethoxyphenyl-prop-2′-enoic acid ester (**17**). Yield = 83%; Yellowish gummy; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 1.28 (bs, 2H, CH_2), 1.33 (s, 3H, CH_3), 1.76 (bm, 2H, CH_2), 2.02 (s, 3H, CH_3), 2.06 (s, 6H, 2xOAc), 1.49, 2.43 (m, 2H, CH_2), 2.40 (bt, 1H, CH), 2.87 (bd, 1H, CH), 3.87 (s, 9H, 2xOCH_3) 4.50 (bs, 1H, CH), 4.93 (s, 1H, CH), 5.32 (s, 1H, CH_2), 5.52 (s, 1H, CH_2), 5.52 (bm, 1H, CH), 6.40 (bs, 1H, CH), 6.52 (d, 1H, CH), 6.67 (d, 1H, CH) 6.80 (s, 2H, CH aromatic), 7.42 (m, 2H, CH aromatic), 7.53 (m, 1H, CH aromatic), 7.57 (d, 1H, CH), 7.87 (m, 2H, CH aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 11.93, 12.94, 14.10, 20.76, 21.40, 22.39, 24.84, 27.05, 29.35, 29.68, 31.91, 33.81, 49.04, 44.88, 47.79, 56.22, 61.00, 63.26, 69.80, 70.70, 74.10, 75.52, 76.09, 105.39, 114.06, 117.68, 128.80, 129.38, 129.48, 129.94, 133.32, 134.40, 139.27, 145.60, 150.88, 153.40, 153.45, 164.38, 169.94, 170.02, Electrospray mass for $C_{43}H_{52}O_{13}$ (MeOH): 779 $[M + Na]^+$.

Brevifoliol-5-O-yl-(4′-methylphenyl)-prop-2′-enoic acid ester (**18**). Yield = 74%; Yellowish gummy; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (s, 3H, CH_3), 1.08 (s, 3H, CH_3), 1.28 (bs, 2H, CH_2), 1.33 (s, 3H, CH_3), 1.95 (bm, 2H, CH_2), 2.00 (s, 3H, CH_3), 2.03 (s, 6H, 2xOAc), 1.72 & 2.32 (m, 2H, CH_2), 2.32 (s, 3H, CH_3), 2.93 (bt, 1H, CH), 2.94 (bd, 1H, CH), 4.50 (bs, 1H, CH), 4.72 (s, 1H, CH), 5.26 (s, 1H, CH_2), 5.50 (s, 1H, CH_2), 5.55 (bm, 1H, CH), 6.03 (bs, 1H, CH), 6.52 (d, 1H, CH), 7.30 (d, 1H, CH), 7.37 (dd, 2H, CH aromatic), 7.40 (dd, 2H, CH aromatic), 7.42 (m, 2H, CH aromatic), 7.49 (d, 1H, CH), 7.62 (m, 1H, CH aromatic), 7.85 (m, 2H, CH aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 11.82, 12.82, 20.61, 21.26, 22.39, 24.60, 26.95, 29.51, 33.76, 38.84, 44.65, 47.15, 62.76, 70.00, 70.83, 73.87, 75.50, 76.21, 106.50, 113.65, 116.65, 128.10, 128.60, 129.18, 129.30, 129.34, 131.70, 133.10, 133.13, 143.00, 145.00, 149.00, 154.24, 164.10, 166.84, 169.80, 169.87, 170.20; Electrospray mass for $C_{41}H_{48}O_{10}$ (MeOH): 723 $[M + Na]^+$.

2.1.3 | UPLC analysis of compound 13

The purity analysis of compound **13** was performed by reverse phase UPLC (ACQUITY UPLC H-Class Bio System; Waters) using C-18 (BEH 130 Å, 1.7 × 50 mm,



1: Brevifoliol

2: R=CH_3-CH=C(CH_3)-; **8:** R=3,5,-Dinitrophenyl; **14:** R=4-Trifluoromethylbenzyl;
3: R=4-Ethylphenyl; **9:** R=4-Methoxybenzyl; **15:** R=4-Aminobenzyl;
4: R=4-Methoxyphenyl; **10:** R=2,4-Dimethoxybenzyl; **16:** R=4-Methoxycinnamyl;
5: R=3,4,5-Trimethoxyphenyl; **11:** R=3,4,5-Trimethoxybenzyl; **17:** R=3,4,5-Trimethoxycinn;
6: R=3,4-Methylenedioxyphenyl; **12:** R=4-Chlorobenzyl;amyl; **18:**R=4-Methylcinnamyl;
7: R=3-Chlorophenyl; **13:** R=4-Nitrobenzyl;

SCHEME 1 Reagent and conditions: (i) Carboxylic acid, DCC, DMAP, CH_2Cl_2, RT, 3–7 hr, 43%–87%

1.7 μm; Waters) at 35 ± 0.1°C constant column temperature, gradient elution of water (0.1% HCOOH) and acetonitrile (0.1% HCOOH) at flow rate of 0.30 ml/min to complete within 5 min from 10% to 90% B. The injection volume was 3.0 μl. The purity is reported based on peak area normalization method.

2.2 | Biological evaluation

2.2.1 | Cytotoxicity evaluation

The assay was performed by MTT assay as per our reported method (Khwaja et al., 2018).

2.2.2 | Soft agar colony formation assay

The was performed against PC-3 cells as per reported method (Kakuguchi et al., 2010).

2.2.3 | Cell cycle analysis

The effect of compound **13** on cell division cycle in PC-3 cells was assessed by flow cytometry with PI-stained cellular DNA, as described earlier (Riccardi & Nicoletti, 2006).

2.2.4 | Annexin V-FITC assay

Annexin V-FITC apoptosis assay by Flow cytometry was done as per reported method (Looi et al., 2013).

2.2.5 | Caspase-3 inhibition assay

The assay was performed in caspase-3 human ELISA kit as per the protocol described from MyBioSource (Catalog # MBS260710) (Harrington, Ho, Ghosh, & Tung, 2008).

2.2.6 | Molecular docking studies

Molecular docking studies of compounds **13** and **16** were performed by docking software AutoDock Vina (Trott & Olson, 2010). The protein 3D crystallographic structures of Caspase-3 PDB ID: 3KJF and Caspase-9 PDB ID: INW9 (Shiozaki et al., 2003) were downloaded from the RCSB PDB database in the PDB format.

2.2.7 | In-vivo efficacy by Ehrlich ascites carcinoma

Ehrlich Ascites Carcinoma (EAC) evaluation of compound **13** was done as per Khwaja et al. (2018). The study was approved by the Institutional Animal Ethics Committee (IAEC) of CSIR-CIMAP, Lucknow, India via CIMAP/IAEC/2016-19/32 dated 09–02–2017.

2.2.8 | Safety studies by acute oral toxicity

Acute oral toxicity of compound **13** was carried out in Swiss albino mice at different oral doses, 5, 50, 300, and 1,000 mg/kg body weight. Experiment was conducted in accordance with the Organization for Economic Co-operation and Development (OECD) test guideline No 423 (Allan, Damodaran, Deshmukh, Goudar, & Ami, 2007 and following the IAEC approved protocols vide ref. No. CIMAP/IAEC/2016-19/01, dated 09/2/2017.

2.2.9 | Statistical analysis

The MTT, Soft agar colony formation, cell cycle, and Annexin V FITC experiments were performed in duplicates, and results are expressed as Mean ± *SD*. For multiple comparisons, each value was compared by one-way ANOVA

following Dunnett's test, Student's *t* test, and Tukey's test in GraphPad Instat version 3.06.

3 | RESULTS

3.1 | Synthesis of C5- esters of brevifoliol

Brevifoliol was our starting substrate. Brevifoliol (1.83 g) was further modified to semi-synthetic derivatives by esterifying through Steglich esterification reaction at C5 hydroxyl (C5-OH) as represented in Scheme 1. Brevifoliol possesses three hydroxyl groups at C5, C13, and C15 on its complex caged structure, that is, allylic hydroxyl at C-13, secondary hydroxyl at C-5 and tertiary hydroxyl at C-15. It was stable naturally in the form of twist-boat/chair form, and this stereochemistry was playing major role in product formation at C-5 and C-13 position. Steglich reaction of brevifoliol by using DCC and DMAP afforded a mixture of derivatives at C-5 and C-13 in a ratio (3:1). However, by decreasing the reaction time and molar ratios of reagents, the product was almost exclusively C5-ester. We used diverse carboxylic acids like benzoic acids (4-ethylbenzoic acid, p-anisic acid, 3,4,5-trimethoxybenzoic acid, piperonylic acid, 3-chlorobenzoic acid, 3,5-dinitrobenzoic acid) phenylacetic acids (4-methoxyPAA, 3,4-dimethoxyPAA, 3,4,5-trimethoxy and cinnamic acids for estrification with brevifoliol. All the esters were purified through column chromatography, and structures were confirmed by spectroscopy (Supporting information).

3.2 | Purity profile of compound 13

Compound **13** had UV maxima at 259 nm, while other minor eluents (reaction impurities) at 228, 263, and 239 nm. Therefore, λ_{max} 245 nm for selected for peak normalization and chromatographic peak integration. However, data acquisition was performed by PDA (190–400 nm) to monitor possible co-elution of neighboring component and peak purity calculation. As a reference method, the functions for peak purity analysis in the chromatographic data processing by chromatography data software (Empower®; Waters) were applied. Under optimum chromatographic conditions, normalized peak area brevifoliol ester **13** (t_R 3.197 min) was defined as purity (>96%).

3.3 | Biological evaluation

3.3.1 | Cytotoxicity

Brevifoliol esters **2–18** were evaluated against a panel of human cancer cell lines, that is, MCF-7 (Breast), COLO-205 (Colon), PC-3 (Prostate), A459 (Lung) and normal cell lines CHANG (Liver) by MTT assay. Out of eighteen compounds, sixteen compounds exhibited cytotoxicity. Five compounds showed significant toxicity against PC-3 cells. Compound **13** (IC_{50} = 4.72 μM), the most active derivative of the series showed selective cytotoxicity against prostate cancer with a high selectivity index (>23.8). However, another compound **16**, also exhibited good cytotoxicity (IC_{50} = 5.02 μM) against PC-3 cells with high selectivity index of >29 (Table 1).

TABLE 1 In-vitro cytotoxicity evaluation of brevifoliol derivatives by MTT assay

Compound no.	Cytotoxicity IC_{50} (μM)					Selectivity index IC_{50} (PC-3)/IC_{50} (CHANG)
	MCF-7	COLO-205	PC-3	A549	CHANG	
1; Brevifoliol	149.58	136.96	NA	132.46	>150	–
2	NA	23.91	61.39	NA	>150	>2.44
3	NA	33.23	NA	NA	>150	–
5	NA	89.58	NA	NA	>150	–
7	NA	NA	71.80	NA	>150	>2.09
8	NA	NA	49.34	NA	>150	>3.04
9	38.68	NA	20.54	NA	>150	>7.30
10	NA	58.59	29.78	33.76	134.25	04.51
11	NA	NA	28.92	33.28	48.15	01.66
12	NA	NA	21.32	NA	>150	>7.04
13	NA	NA	**4.72**	NA	**112.34**	**23.80**
14	NA	76.05	32.08	56.78	66.60	2.08
15	NA	128.54	19.52	36.48	144.60	7.41
16	NA	47.29	**5.02**	NA	>150	**>29.88**
17	NA	56.04	NA	NA	>150	–
18	NA	62.34	22.47	NA	>150	>6.68
Doxorubicin	3.90	2.71	5.19	3.08	30.09	**5.79**

Note: IC_{50} > 150 μM was considered as NA (inactive); Bold values shows efficacious compounds.

TABLE 2 Effect of brevifoliol derivative **13** on colony formation in PC-3 cells in soft agar after 24h incubation (no. of seeded cells = 5×10^4 cells/ml, area of 60 mm plate = 2,826 mm^2)

Condition	Concentration (μg/ml)	Average % live cells[a]	PC-3 (% dead cells)	PC-3 IC_{50} (μg/ml)
Control	–	100	0	
Compd. **13**	2	77.58	22.42 ± 3.77**	9.59 ± 1.85
	10	52.59	47.41 ± 3.73**	
	50	30.82	69.18 ± 0.52**	

[a]Number of colonies = 10,675 ± 333; $n = 2$.

**$p < .01$ (One-way ANOVA Dunnett's test).

3.3.2 | Soft agar colony formation assay

It is a semi-quantitative measure of the morphological transformation of cell colonies induced by the test compound such as loss of contact inhibition (cells can grow over one another) and anchorage independence (cells form colonies in soft agar). Compound **13** suppressed the growth of colonies of PC-3 cells by 22%–69% at 2–50 μg/ml concentrations with an IC_{50} of 9.59 μg/ml. It clearly indicates potential antiproliferative action of compound **13** which is quite significant and also concentration dependent (Table 2).

3.3.3 | Cell cycle analysis

Compound **13** was evaluated for its effect on cell cycle phases in PC-3 cells. At IC_{50} for 24 hr, poor S phase arrest was observed, but significant G2/M phase arrest. While at $2xIC_{50}$ value, there was significant arrest in S phase and G2/M phase arrest was nominal. Further, compound **13** induced apoptosis significantly at $2 \times IC_{50}$ (Figure 2).

3.3.4 | Apoptosis induction by Annexin V FITC

Compound **13** induced late apoptosis by 4.3% and necrosis by 3.4% at its half IC_{50} (1.95 μM). It did not display early apoptosis. At higher concentration (IC_{50}, 4.72 μM), apoptosis was reduced to 2.4%, while necrosis was enhanced to 3.9% (Figure 3).

3.3.5 | Apoptosis induction by caspase pathway

Caspase-3 plays an important role in the induction of apoptosis through both intrinsic (mitochondrial) and extrinsic (death ligands) pathways. Compound **13** activated caspase-3 moderately in PC-3 cells (Table 3). There was no activation at IC_{50}, but at $4xIC_{50}$, there was significant effect on caspase-3 activation (26.6%) but much lower than doxorubicin which exhibited higher effect (36.7% and 51.4%) at 1 and 2 μM.

3.3.6 | Molecular docking studies

Both the brevifoliol derivatives **13** and **16**, and standard drug doxorubicin occupied the same binding pocket (Figure 4) of caspase-3 (seven common amino acids) and showed binding energy of −8.0, −7.7, and −8.1 kcal/mol (Table SA, Supporting information). Similarly, with caspase-9, these ligand showed docking energy of −7.9, −7.7, and −7.4 kcal/mol (ten common amino acids) (Table SB, Supporting information).

The calculated "Lipinski's rule of five" showed that both the compounds violated two of the rules of five and showed higher molecular weight, more than 10 H-bond acceptors (Table 4). However, it cannot be a strict rule as in last three years FDA-USA approved 21% of new oral drugs beyond the rule of 5 due to high efficacy for the treatment of HCV and cancer (Degoey, Chen, & Cox 2018).

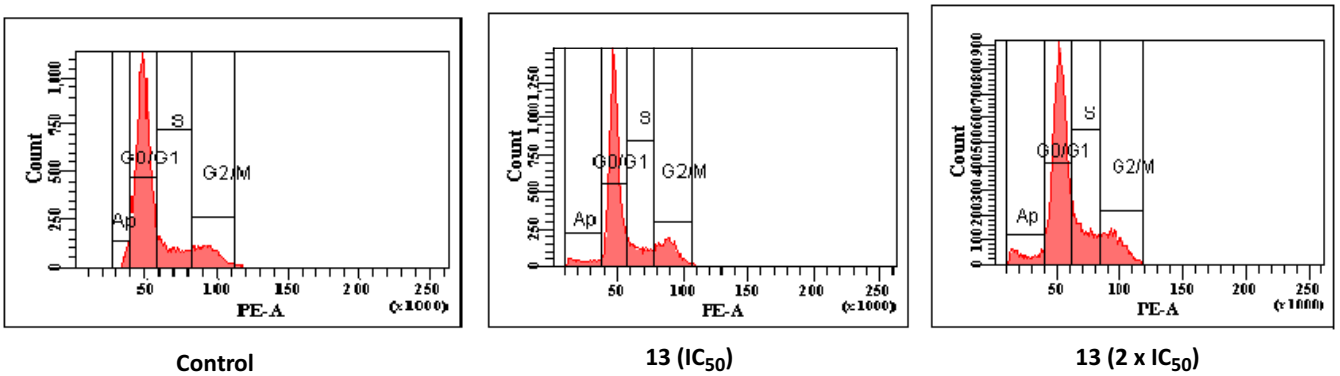
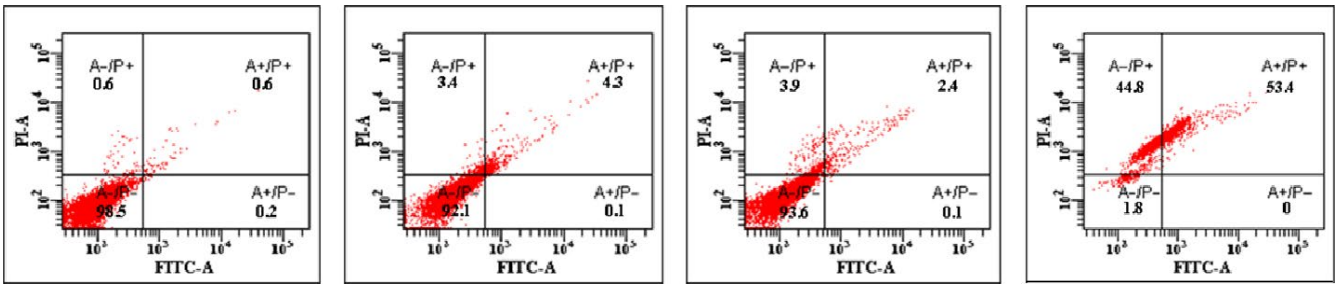


FIGURE 2 Cell cycle analysis of compound **13** in PC-3 cells [Colour figure can be viewed at wileyonlinelibrary.com]



(a) Control **(b)** **13** (1.95 μM) **(c)** **13** (3.89 μM) **(d)** Doxorubicin (1 μM)

FIGURE 3 Induction of apoptosis and necrosis by compound **13** in PC-3 cells by Annexin V-FITC assay [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Activation of caspase-3 by ester derivative **13**

S. No.	Compound	Concentration (μM)	Caspase-3% activation
1.	13	3.90	0.00
2.	13	15.60	26.61[a]
3.	Doxorubicin	1.00	36.73
4.	Doxorubicin	2.00	51.37

[a]Non-significant change was observed compared to Doxorubicin using One-way ANOVA Dunnett's test.

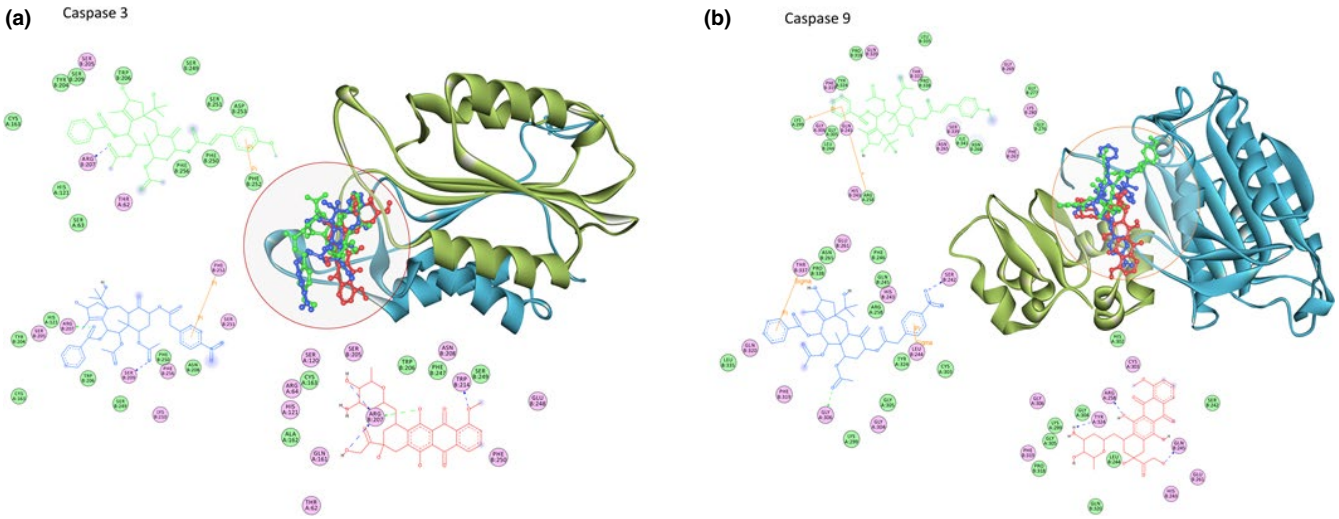


FIGURE 4 (a) Compounds **13** (Blue), **16** (Green), and doxorubicin (Red) docked in the same binding pocket of caspase-3 PDB ID: 3KJF and (b) caspase-9 PDB ID: INW9 [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 4 Drug likeness (oral bioavailability) of compounds **13** and **16** through Lipinski's rule of five

Compound	Molecular weight (≤500)	LogP (≤5)	H-bond donors (≤5)	H-bond acceptors (≤10)	Rule of five violations allowed (≤1)
Compound **13**	720.782	4.28	3	13	2
Compound **16**	716.813	4.91	2	11	2
Doxorubicin	541.546	0.43	7	11	3

3.3.7 | In-vivo efficacy by Ehrlich ascites carcinoma (EAC)

In EAC experiment of compound **13** showed moderate activity. There was no loss in body weight of animals (Table 5). Compound **13** reduced EAC tumor by 36.67%, 44.51%, 47.35%, and 55.85% at 25, 50, 75, and 100 mg/kg intraperitoneal doses, respectively (Table 6). There was no mortality in any of the experimental group. However, the efficacy of compound **13** was much lower than the standard drug 5-fluorouracil.

3.3.8 | Safety studies

In acute oral toxicity, no significant changes were observed in all the parameters studied like morbidity, mortality, observational parameters, body weight, serum biochemical parameters, organ weight, and most of the hematological parameters up to the dose level of 1,000 mg/kg body weight (Table 7, Figure 5), differential leukocyte count showed significant changes in the treated groups compared to control. Lymphocyte and eosinophil count showed significant changes in group of mice treated with compound at 1,000 mg/kg compared to control (Figure 6).

TABLE 5 Effect of compound **13** on body weight of mice bearing Ehrlich ascites carcinoma

	Body weight (g)				
Sample	**Dose**	**Day 1**	**Day 5**	**Day 9**	**Day 12**
Control	NS (0.2 ml), i.p.	23.42 ± 1.29	26.12 ± 0.92	32.12 ± 2.4	35.04 ± 1.52
Compound **13**	25 mg/kg, i.p.	23.40 ± 1.28	26.40 ± 0.92	30.52 ± 1.23	30.88 ± 1.38
	50 mg/kg, i.p.	23.60 ± 0.81	25.94 ± 0.46	30.10 ± 0.61	31.20 ± 0.71
	75 mg/kg, i.p.	22.80 ± 1.15	25.62 ± 0.93	30.62 ± 1.38	31.24 ± 1.54
	100 mg/kg, i.p.	22.80 ± 0.96	25.04 ± 0.47	29.82 ± 1.20	30.52 ± 1.62
5-Fluorouracil	20 mg/kg, i.p.	21.50 ± 0.57	24.02 ± 0.77	21.05 ± 1.61	20.70 ± 1.75

TABLE 6 In-vivo efficacy of compound **13** against Ehrlich ascites carcinoma

Sample	**Dose (mg/kg) i.p.**	**Tumor volume (ml) Mean ± *SE***	**Tumor weight (g) Mean ± *SE***	**Tumor cell count (1×10^7) Mean ± *SE***	**Tumor growth inhibition (%)**
Control	NS (0.2 ml), i.p.	7.50 ± 0.74	7.81 ± 0.70	57.75 ± 9.74	0
Compound **13**	25 mg/kg, i.p.	5.10** ± 0.98	5.28** ± 0.99	36.75** ± 2.89	36.67
	50 mg/kg, i.p.	3.90** ± 1.05	4.30** ± 0.94	32.05** ± 1.47	44.51
	75 mg/kg, i.p.	5.40* ± 1.22	5.72* ± 1.19	30.40** ± 3.65	47.35
	100 mg/kg, i.p.	6.92 ± 1.60	7.05 ± 1.58	25.50** ± 2.19	55.85
5-Fluorouracil	20 mg/kg, i.p.	1.51** ± 0.50	1.70** ± 0.52	9.56** ± 4.74	83.54

**$p < .01$;
*$p < .05$ (One-way ANOVA Dunnett's test).

TABLE 7 Effect of compound **13** on body weight, hematological, and serum biochemical parameters in Swiss albino mice

	Dose of compound 13 at mg/kg body weight as a single oral dose				
Parameters	**Control**	**5 mg/kg**	**50 mg/kg**	**300 mg/kg**	**1,000 mg/kg**
Body weight (g)	35.64 ± 0.73	33.77 ± 2.43	33.51 ± 1.41	32.06 ± 1.47	31.02 ± 13.87
Hemoglobin (g/dl)	11.01 ± 0.83	11.79 ± 0.31	11.94 ± 0.73	10.95 ± 0.66	12.13 ± 0.72
RBC (million/mm^3)	5.40 ± 0.56	3.78 ± 0.20	4.09 ± 0.52	5.07 ± 0.39	3.76 ± 0.32
WBC(thousands/mm^3)	6.81 ± 1.24	4.91 ± 0.45	5.20 ± 0.60	4.50 ± 0.51	7.95 ± 1.11
ALP (U/L)	298.35 ± 29.67	235.31 ± 50.26	339.00 ± 19.80	342.76 ± 23.53	309.35 ± 68.60
SGOT (U/L)	41.02 ± 6.37	44.98 ± 7.19	53.04 ± 8.91	43.82 ± 6.06	52.14 ± 5.00
SGPT (U/L)	35.20 ± 6.65	31.36 ± 4.84	24.65 ± 4.25	28.45 ± 6.82	25.40 ± 5.30
Creatinine (mg/dl)	1.61 ± 0.35	1.71 ± 0.62	3.04 ± 0.61	1.82 ± 0.35	2.03 ± 0.45
Triglycerides (mg/dl)	67.99 ± 9.46	52.85 ± 3.03	81.81 ± 10.66	61.63 ± 7.59	57.16 ± 5.73
Bilirubin(mg/dl)	0.67 ± 0.03	0.87 ± 0.07	0.72 ± 0.10	0.67 ± 0.06	0.64 ± 0.05
Cholesterol (mg/dl)	55.87 ± 6.97	39.10 ± 7.63	34.26 ± 3.36	57.84 ± 8.69	54.65 ± 3.23
Albumin(g/dl)	3.66 ± 0.33	3.04 ± 0.33	2.19 ± 0.13	3.25 ± 0.20	4.02 ± 0.66
Protein(mg/ml)	1.60 ± 0.24	2.17 ± 0.14	2.22 ± 0.09	1.95 ± 0.10	1.99 ± 0.15

Note: Mean ± *SE*; $n = 6$.
*$p < .05$ compared to control.

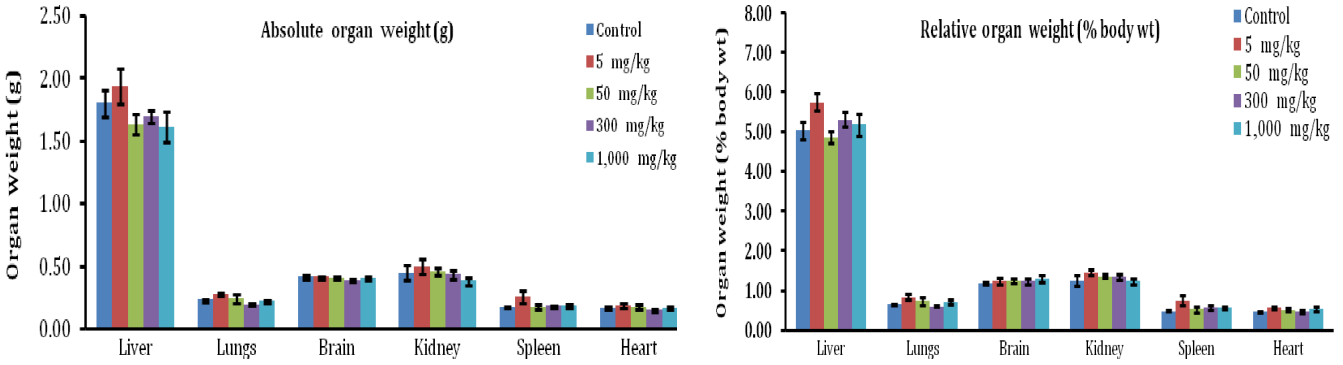


FIGURE 5 Effect of compound **13** on absolute and relative organ weight in Swiss albino mice (mean ± *SE*; $n = 6$, *, $p < .05$ compared with control) [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

The incidence of prostate cancer has been rising around the globe in the recent years. The etiology of the disease is quite puzzling. There was a strong correlation between ages, with increased relative risk for individuals with a family history (Albright et al., 2014). The current challenges of the field include lack of suitable animal models. Brevifoliol derivatives **13** and **16** exhibited potential antiproliferative activity (IC_{50} = 4.7 μM & 5 μM) against PC-3 cell line. PC-3 and DU-145 are hormone-(Androgen receptor, AR) independent prostate cancer cell lines while, LNcaP is an AR hormone-dependent prostate cancer cell line. In soft agar colony assay, compound **13** exhibited concentration dependent inhibition of prostate cancer cell colonies. Soft agar colony assay is a gold standard assay for cellular transformation or tumorigenicity in-vitro. The growth in soft agar is strongly correlated with tumorigenicity in animals, typically mouse xenografts (Rotem et al., 2015).

In cell cycle analysis, compound **13** induced both S-phase and G2/M phase arrest in concentration dependent manner. However, at double IC_{50} cell cycle arrest was quite prominent with significant apoptosis induction. The cell cycle arrest at S-phase might be due to induction of apoptosis through caspase pathway which was moderately effective in caspase-3 activation assay. Caspases are cysteine aspartic proteases playing an important role in apoptosis and inflammation (Goodsell, 2000). Among these, caspase-9 as initiator caspase and caspase-3, as executioner caspase are activated in the both intrinsic and extrinsic caspase cascade pathways to initiate apoptosis (Wilson & Kumar, 2018). Avoidance to programmed cell death is considered one of the important hallmarks of cancer (Hanahan & Weinberg, 2011). Therefore, induction of apoptosis in cancer cells is one of the most successful approaches to treat cancer. However, induction of cell cycle arrest also at G2/M phase by compound **13**, could not be understood, it needs to be further elaborated. There might be some other mechanistic pathway which affects later phase of cell cycle division of PC-3 cells. It will be worth mentioning here that in our previous experiments of tubulin kinetics, brevifoliol (**1**) as such did not exhibit any antitubulin effect. In general antitubulins exhibit G2/M phase arrest in cell cycle (Negi et al., 2015).

Compound **13** exhibited moderate efficacy in Ehrlich ascites carcinoma. It reduced 55.85% of EAC tumor at 100 mg/kg dose. EAC is an undifferentiated carcinoma, hyperdiploid, rapid proliferation, 100% malignancy, and shorter life span. EAC tumor cells are much more difficult to break than most nonmalignant somatic cells (Bearns & Kessel, 1968).

Drug safety studies are a key component to the Investigational New Drugs (INDs). It mainly points-out the adverse drug reactions (ADRs) on exposure of investigational drug to experimental animals. ADR is considered as to be fourth and sixth cause of deaths in USA. Overall, brevifoliol derivative **13** did not show any toxicity against most of the experimental parameters in acute oral toxicity. It was well tolerated by Swiss albino mice. However, at higher dose (1,000 mg/kg), there were some minor issues with SGOT level and RBC count but non-significant. Nevertheless, long-term safety studied will be required at sub-acute and chronic levels for further development of this investigational lead compound.

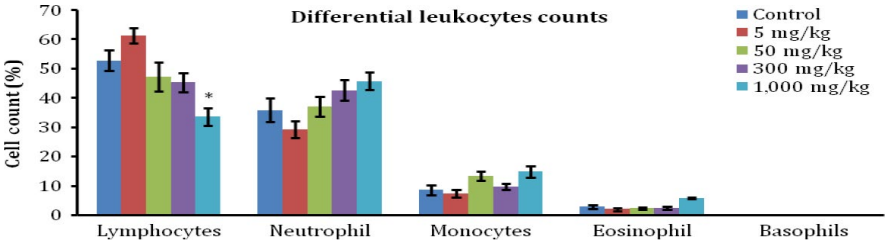


FIGURE 6 Effect of Compound **13** on differential leukocytes counts in Swiss albino mice (mean ± *SE*; $n = 6$, *, $p < .05$ compared with control) [Colour figure can be viewed at wileyonlinelibrary.com]

5 | CONCLUSIONS

In conclusion, C5 modification of brevifoliol has yielded a potent antiproliferative derivative, that is compound **13** against prostate cancer. It exhibited anticancer activity by apotosis induction in PC-3 cells via activation of caspase cascade pathway. Compound **13** was moderately effective in reduction of Ehrlich ascites carcinoma in rodents. Further, it was safe and well tolerated in Swiss albino mice up to 1,000 mg/kg dose. Further optimization of this lead compound is underway in our laboratory.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data have been peer-reviewed.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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