Med Chem Res
DOI 10.1007/s00044-017-1949-z

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

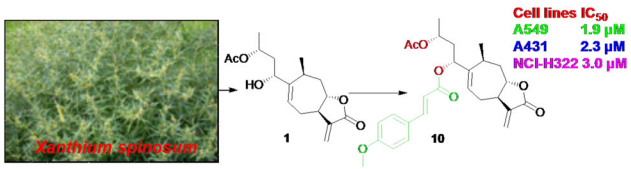
Isolation of isoxanthanol and synthesis of novel derivatives as potential cytotoxic agents

Praveen K. Chinthakindi[1,4] · Santosh K. Rath[1,2] · Jasvinder Singh[2,3] · Shashank Singh[2,3] · Surrinder Koul[1] · Payare L. Sangwan[1,2]

Received: 21 December 2016 / Accepted: 3 June 2017

Abstract Novel synthetic derivatives of sesquiterpene lactone isoxanthanol (**1**) have been prepared and bioevaluated against four human cancer cell lines viz. T98G (glioblastoma), A431 (epidermoid carcinoma), NCI-H322 (bronchioloalveolar carcinoma), and A549 (lung adeno carcinoma) for their cytotoxic potential using paclitaxel as the standard. This has resulted in the identification of potent molecules displaying IC_{50} 1.9 and 5.0 µM, respectively against the A549 cancer cell line. The study has resulted in the identification of potential cytotoxic activity of the analog (compound **10**) bearing electron donating aryl alkenoic substituent. Furthermore, the induction of cell death has been assessed for the most active compound (**10**) using flow cytometric method and sub-G1 cell population determination by propidium iodide staining. The concentration dependent inhibitory effect of **10** on the A549 cells ability did not reproduce and form colonies at 20 µM concentration.

Graphical Abstract Synthesis of isoxanthanol derivatives and their cytotoxic study resulted in identification of potential cytotoxic agents. Compound **10**, one of its aryl alkenoic substituent showed potency against NCI-H322 (bronchioloalveolar carcinoma), and A549 (lung adeno carcinoma) cell lines.



Keywords Isoxanthanol · Sesquiterpene · Lactone · Human leukemia

Electronic supplementary material The online version of this article (doi:10.1007/s00044-017-1949-z) contains supplementary material, which is available to authorized users.

✉ Surrinder Koul
skoul@iiim.ac.in

✉ Payare L. Sangwan
plsangwan@iiim.ac.in

[1] Bioorganic Chemistry Division, CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu 180001, India

[2] Academy of Scientific and Innovative Research (AcSIR), CSIR-IIIM Campus, Jammu, India

[3] Cancer Pharmacology Division, CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu 180001, India

[4] Present address: Catalysis and Peptide Research Unit, University of Kwazulu-Natal, Durban, South Africa

Abbreviations

NP's	Natural products
SLs	Sesquiterpene lactones
NCEs	New chemical entities
FDA	Food and drugs administration
J	Coupling constant
HRMS	High resolution mass spectra
PBS	Phosphate buffer saline
SRB	Sulforhodamine B
TCA	Trichloroacetic acid
DMAP	*N*,*N*-dimethyl amino pyridine
CC	Column chromatography

Published online: 22 June 2017

Introduction

Natural products (NP's) are compounds that are commonly found in nature, having a wide range of pharmacophores, high degree of stereochemistry and possess various biological applications that serve as an excellent source of lead scaffolds in drug discovery program (Harvey et al. 2015). There are only a few instances, where NPs have been used as drugs as such e.g., vinblastine, vincristine, paclitaxel, camptothecin, colchicine, and silvestrol etc. In the majority of the cases, the drugs have been developed in the form of structural analogs and/or derivatives of NPs as a base skeleton e.g., flavopiridol, etoposide, teniposide, combretastatin A-4 phosphate, CDDO, topotecan, irinotecan, vinorelbine, vindesine, minnelide etc. This is well corroborated by the fact that, in recent years Food and drugs administration approved new chemical entities, have only 8% of NPs compared to the NP derivatives (Cragg et al. 2014; Newman and Cragg 2012), which are three times higher. Realistically, the structural modification of NPs has been undoubtedly playing pivotal role in the development of efficient modern medicine. Further docking studies support in identification of potent molecules (Taha et al. 2016, 2017).

Natural sesquiterpene lactones (SLs) and their derivatives are of great interest due to their vast pharmacological properties and this asset has attracted many medicinal chemists for further tailoring and screening them as novel therapeutic agents. Many of these SLs are in cancer clinical trials and also selectively targeting tumor and cancer stem cells while leaving normal cells untouched (Ghantous et al. 2010, 2013; Jordan 2006; Gach and Dalugosz 2014; Zhang et al. 2012; Christensen et al. 2009). However, poor cell permeability and solubility is a major concern for SLs due to their extensive plasma protein interactions and hydrophobic nature. In addition to that, some of them having allergenic properties and off-target effect (Ghantous et al. 2010; Efferth 2006; Zhou and Zhang 2008; Schmidt 1997). These issues might be overcome by chemical modification and pro drug approach.

So far there is no detailed anticancer report on isoxanthanol (Nour et al. 2009; Favier et al. 2005; Yoon et al. 2008; Vasas and Hohmann 2011). Isolation of isoxanthanol has been problematic from *Xanthium* species (Abdei-Mogib et al. 1991; Omar et al. 1984; Bohlmann et al. 1982) by column chromatography (CC) due to its isomerization to xanthanol (**1a**) (Fig. 1) and presuming that, this may be one of the reasons that no literature reports are present with respect to its chemical modification. Therefore natural product isoxanthanol (**1**) was explored for chemical modification to investigate new hit or lead molecule for cancer as a part of our ongoing research activities (Khan et al. 2016; Chinthakindi et al. 2013, 2017; Dangroo et al. 2017; Dar et al. 2016; Gupta et al. 2016; Majeed et al. 2012, 2013) in the area of NP's-based medicinal chemistry.

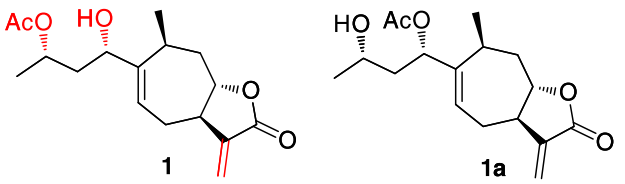


Fig. 1 Active sites (shown in *red*) of isoxanthanol (**1**) and xanthanol (**1a**) (color figure online)

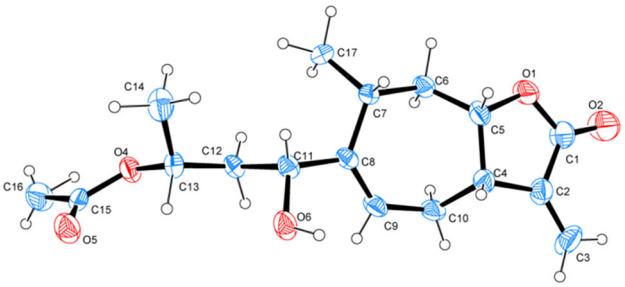


Fig. 2 The ORTEP diagram indicates numbering scheme and the molecular conformation of isoxanthanol in crystals. The displacement ellipsoids are drawn at the 40% probability level. H atoms are shown as small spheres of arbitrary radii

In the present study, the NP was isolated by repeated CC and re-crystallization process (see experimental data) in moderate yields (0.12% from 1 Kg) from the areal part of *Xanthium spinosum* plant. The absolute stereochemistry of the isoxanthanol established through X-ray crystallography see Fig. 2. The chemical modification of **1** was carried out for the identification of best possible potent antiproliferative analog.

Materials and methods

Reagents and instruments

All reagents for chemical synthesis were obtained from Sigma Aldrich and the solvents used in reactions were distilled and dried prior to use. All chemical reactions were monitored by thin layer chromatography (TLC) on 0.25 mm silica gel 60 F254 plates (E. Merck) using 2% ceric ammonium sulfate solution for detection of the spots. Purification of compounds was carried out by CC using silica gel 60–120 mesh stationary phase. All nuclear magnetic resonance (NMR) (^{1}H NMR and ^{13}C NMR) spectra were recorded on Bruker DPX 200, 400, and DPX 500 instruments using $CDCl_3$ as the solvents with tetramethylsilane as internal standard. Chemical shifts are expressed in δ and coupling constants (J) in Hertz. High resolution mass spectra (HRMS) were recorded on Agilent

Technologie 6540 instrument and infrared (IR) was recorded on an fourier transform infrared Bruker (270–30) spectrophotometer.

RPMI-1640 medium, MEM medium, penicillin, streptomycin, sodium bicarbonate, phosphate buffer saline (PBS), sulforhodamine B (SRB), trypsin, rhodamine-123, paclitaxel and dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich. Propidium iodide, Rnase, trichloroacetic acid (TCA) were procured from Merck. Glacial acetic acid was purchased from Fisher Scientific. Ethylenediaminetetraacetic acid was purchased from Himedia. Fetal bovine serum was from Gibco and erlotinib was from Selleck Chemicals.

Cell culture, growth conditions and treatment conditions

Human glioblastoma cancer cell line (T98G), lung carcinoma (A549, NCI-H322) and epidermal carcinoma (A431) were procured from European collection of cell culture. Cells were grown in (RPMI-1640/ MEM) medium supplemented with 10% fetal bovine serum, 2 mg/ml sodium bicarbonate, 100 µg/ml streptomycin and 100 units/ml penicillin in carbon dioxide incubator (Thermo Scientific, USA) at 37 °C, 5% CO_2 and 98% RH. Cells were treated with different compounds dissolved in DMSO with untreated control receiving only vehicle (DMSO).

Isolation of isoxanthanol

Isoxanthanol (**1**) was isolated from the aerial part of *Xanthium spinosum* (duly authenticated by the Taxonomist of our institute) by repeated CC over silica gel 60–120 mesh, and purified by recrystallization from chilled ether. The NP was chemically characterized by spectral data analysis and found in agreement with the literature data (Abdei-Mogib et al. 1991; Omar et al. 1984; Bohlmann et al. 1982; Marco et al. 1993) and finally its absolute configuration confirmed by X-ray crystallography (see Fig. 2). This is the first report of the establishment of absolute stereochemistry by X-ray analysis.

*(2S,4S)-4-hydroxy-4-((3aR,7S,8aS)-7-methyl-3-methylene-2-oxo-3,3a,4,7,8,8a-hexahydro-2H-cyclohepta[b]furan-6-yl)butan-2-yl acetate (Isoxanthanol) (**1**)*

The compound was obtained as white crystalline; mp 101.5 °C; $[\alpha]^{25D}$ −27.2 (c 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 6.27$ (1H, d, $J = 3.2$ Hz, CH_2=C–), 5.73 (1H, dd, $J = 5.6$, 9.2 Hz,=CH–CH_2–), 5.52 (1H, d, $J = 2.8$ Hz, –CH_2=C–), 4.96 (1H, dt, $J = 6.0$, 12.4 Hz, –CHOCO–), 4.61 (1H, ddd, $J = 2.8$, 8.4 Hz, –CHOAc), 4.10 (1H, dd, $J = 5.6$, 8.4 Hz, CHOH), 3.3–4.2 (1H, m, –CHCH–), 2.56–2.66 (1H, m, –CH_2CH–), 2.43–2.50 (1H, m, CH_2–CH–), 2.25–2.33 (1H, m, CH–CH_2–), 2.04 (3H, s, CH_3CO–), 1.78–1.88 (1H, m, CH–CH_2–), 1.63–1.77 (2H, m, CH–CH_2–), 1.56–1.62 (1H, m, CH_2–CH_2–), 1.28 (3H, d, $J = 6.4$ Hz, CH_3–CH–), 1.19 (3H, d, $J = 6.8$ Hz, CH_3–CH–); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 170.67$ (<u>C</u>=O), 170.18 (<u>C</u>=O), 147.67 (CH=<u>C</u>), 138.58 (CH_2=<u>C</u>), 122.44 (<u>CH_2</u>=C), 122.16 (<u>CH</u>=C), 78.96 (<u>CH</u>–O), 73.61 (<u>CH</u>–OAc), 69.32 (<u>CH</u>–OH), 42.10 (O–CH–<u>CH_2</u>–CH–O), 41.36 (CH_2–<u>CH</u>–CH–O), 36.96 (CH–<u>CH_2</u>–CH–O), 32.83 (=CH–<u>CH_2</u>–CH), 26.00 (=C–<u>CH</u>–CH_3), 22.09 (CO–<u>CH_3</u>), 21.42 (<u>CH_3</u>–CH–O), 20.38 (<u>CH_3</u>–CH); IR (neat) γ_{max} 3467, 2955, 2934, 2872, 1761, 1736, 1658, 1459, 1409, 1373, 1328, 1247, 1209, 1124, 1089, 1049, 1015, 979, 856, 834, 778, 749, 657, 585, 541, 512 cm^{-1}; HRESIMS *m/z* (pos): 331.1522 $C_{17}H_{23}NaO_5$ (calcd. 331.1522).

General procedure for preparation of acyl derivatives of isoxanthanol (2–6)

Compounds **2–6** were synthesized by treating isoxanthanol (**1**) with appropriate alkanoic anhydride in presence of catalytic amount of *N*, *N*-dimethyl amino pyridine (DMAP) and the progress of reaction monitored by TLC. On completion of the reaction, the contents poured in ice water and the oily material separated and dissolved in dichloromethane. The organic layer washed with water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The acyl derivatives thus obtained were purified by CC over silica gel 60–120 mesh, using hexane: ethyl acetate mixture (49:1) as an eluent to give compounds **2–6** in >90% over all yield. The spectral data of all the derivatives are given below.

*(1S,3S)-1-((3aR,7S,8aS)-7-methyl-3-methylene-2-oxo-3,3a,4,7,8,8a-hexahydro-2H-cyclohepta[b]furan-6-yl) butane-1,3-diyl diacetate (**2**)*

Yellowish gummy mass (This compound was prepared by reacting 100 mg isoxanthanol (**1**) with 33 µL acetic anhydride and purified by the procedure as described above to obtained 106 mg with 97% yield); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 6.26$ (1H, d, $J = 3.2$ Hz, CH_2=C–), 5.81 (1H, dd, $J = 5.6$, 9.2 Hz,=CH–CH_2–), 5.52 (1H, d, $J = 2.8$ Hz, –CH_2=C–), 5.08 (1H, dd, $J = 5.6$, 8.4 Hz, CHOAc), 4.88 (1H, dt, $J = 6.0$, 12.4 Hz, –CHOCO–), 4.62 (1H, ddd, $J =$ 2.8, 8.4 Hz, –CHOAc), 3.28–2.42 (1H, m, –CH_2CH–), 2.55–2.69 (1H, m, CH_2–CH–), 2.39–2.2.52 (1H, m, CH–CH_2–), 2.23–2.37 (2H, m, CH & CH_2), 2.04 (2 × 3H each, s, CH_3–CO–), 1.84 (1H, m, CH_2–CH_2–), 1.75 (2H, m, CH_2–CH_2–), 1.28 (3H, d, $J = 6.4$ Hz, CH_3–CH–), 1.19 (3H, d, $J = 6.8$ Hz, CH_3–CH–); ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 170.38 (<u>C</u>=O), 170.25 (<u>C</u>=O), 170.00 (<u>C</u>=O), 144.20 (<u>C</u>=CH), 138.63 (CH_2=<u>C</u>), 124.99 (<u>CH_2</u>=C), 122.16 (<u>CH</u>=C), 78.78 (<u>CH</u>–O), 74.98 (<u>CH</u>–OAc), 67.93

(*CH*–OH), 41.33 (O–CH–*CH*$_2$–CH–O), 39.78 (CH$_2$–*CH*–CH–O), 36.94 (CH–*CH*$_2$–CH–O), 33.50 (=CH–*CH*$_2$–CH), 26.18 (=C–*CH*–CH$_3$), 21.64 and 21.31 (2 × CO–*CH*$_3$), 21.30 (*CH*$_3$–CH–O), 19.94 (*CH*$_3$–CH); IR (neat) γ_{max} 3452, 2930, 2873, 1763, 1736, 1660, 1460, 1372, 1328, 1241, 1123, 1088, 1050, 1018, 958, 855, 815, 607, 545, 518, 506 cm^{-1}; HRESIMS *m/z* (pos): 373.1621 $C_{19}H_{26}NaO_6$ (calcd. 373.1623).

*(1S,3S)-3-acetoxy-1-((3aR,7S,8aS)-7-methyl-3-methylene-2-oxo-3,3a,4,7,8,8a-hexahydro-2H-cyclohepta[b]furan-6-yl)butyl butyrate (**3**)*

Yellowish gummy mass (This compound was prepared by reacting 100 mg isoxanthanol (**1**) with 55 µL butyric anhydride and purified by the procedure as described above to obtained 114 mg with 94% yield); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 6.24$ (1H, d, $J = 3.2$ Hz, CH_2=C–), 5.79 (1H, dd, $J = 5.6$, 9.2 Hz, =CH–CH_2–), 5.50 (1H, d, $J = 2.8$ Hz, CH_2=C–), 5.07 (1H, dd, $J = 5.6$, 8.4 Hz, –CH–$OCOCH_2$–), 4.86 (1H, dt, $J = 6.0$, 12.4 Hz, CH–OCO), 4.61 (1H, ddd, $J = 2.8$, 8.8 Hz, CH–OAc), 3.21–3.33 (1H, m, CH–CH–), 2.57–2.67(1H, m, CH_2–CH–), 2.45–2.38 (2H, m, CH_2–CH–), 2.21–2.35 (3H, m, CH_2–CH_2–, CH), 2.03 (3H, s, CH_3–CO–), 1.82 (1H, m, CH_2–CH_2–), 1.73 (2H, m, CH_2–CH_2–), 1.63 (2H, m, CH_2–CH_2–), 1.22 (3H, d, $J = 6.4$ Hz, CH_3–CH–), 1.12 (3H, d, $J = 6.8$ Hz, CH_3–CH–), 0.94 (3H, t, $J = 6.4$ Hz, CH_3–CH_2–); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 172.93$ (*C*=O), 170.37 (*C*=O), 169.98 (*C*=O), 144.47 (*C*=CH), 138.63 (CH_2=*C*), 124.75 (*CH*$_2$=C), 122.11 (*CH*=C), 78.79 (*CH*–O), 74.58 (*CH*–OAc), 67.94 (*CH*–OH), 41.34 (O–CH–*CH*$_2$–CH–O), 39.83 (CH_2–*CH*–CH–O), 36.89 (CH–*CH*$_2$–CH–O), 36.44 (CH_2–*CH*$_2$–C = O), 33.70 (=CH–*CH*$_2$–CH), 26.14 (=C–*CH*–CH_3), 21.56 (CO–*CH*$_3$), 21.25 (*CH*$_3$–CH), 19.83 (*CH*$_3$–CH–O), 18.37 (*CH*$_2$–CH_3), 13.63 (CH_2–*CH*$_3$); IR (neat) γ_{max} 3435, 2963, 2933, 2875, 1764, 1733, 1658, 1459, 1372, 1328, 1244, 1179, 1087, 1050, 1018, 958, 855, 815, 609 cm^{-1}; HRESIMS *m/z* (pos): 401.1939 $C_{21}H_{30}NaO_6$ (calcd. 401.1934).

*(1S,3S)-3-acetoxy-1-((3aR,7S,8aS)-7-methyl-3-methylene-2-oxo-3,3a,4,7,8,8a-hexahydro-2H-cyclohepta[b]furan-6-yl)butyl pentanoate (**4**)*

Yellow liquid (This compound was prepared by reacting 100 mg isoxanthanol (**1**) with 65 µL valeric anhydride and purified by the procedure as described above to obtained 121 mg with 96% yield); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 6.26$ (1H, d, $J = 3.2$ Hz, CH_2=C–), 5.81 (1H, dd, $J = 5.6$, 9.2 Hz, =CH–CH_2–), 5.52 (1H, d, $J = 2.8$ Hz, CH_2=C–), 5.08 (1H, dd, 5.6, 8.4 Hz, CH–$OCOCH_2$–), 4.88 (1H, dt, $J = 6.0$, 12.4 Hz, CH–OCO–), 4.62 (1H, ddd, $J = 2.8$ and 8.8 Hz, CH–OAc), 3.34–3.32 (1H, m, –CH–CH–), 2.65 (1H, m, CH_2–CH–), 2.35 (1H, t, $J = 6.4$ Hz, CH_2–CH–), 2.28–2.33 (2H, m, CH–CH_2–), 2.05 (3H, s, CH_3–CO–), 1.7–1.8 (2H, m, CH_2–CH_2–), 1.65 (2H, m, CH_2–CH_2–), 1.58–1.66 (2H, m, CH_2–CH_2–), 1.52–1.48 (1H, m, –CH_2–CH_2–), 1.3–1.41 (2H, m, –CH_2–CH_2–), 1.24 (3H, d, $J = 6.4$ Hz, CH_3–CH), 1.13 (3H, d, $J = 7.10$ Hz, CH_3–CH–), 0.91 (3H, q, $J = 6.3$ Hz, CH_3–CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz,): $\delta = 173.01$ (*C*=O), 170.33 (*C*=O), 169.91 (*C*=O), 144.23 (*C*=CH), 138.44 (CH_2=*C*), 124.62 (*CH*$_2$=C), 122.02 (*CH*=C), 78.68 (*CH*–O), 74.45 (*CH*–OAc), 67.81 (*CH*–OH), 41.15 (O–CH–*CH*$_2$–CH–O), 39.61 (CH_2–*CH*–CH–O), 36.69 (CH–*CH*$_2$–CH–O), 34.06 (CH_2–*CH*$_2$–C=O), 33.63 (=CH–*CH*$_2$–CH), 25.97 (=C–*CH*–CH_3), 25.50 (–*CH*$_2$–CH_2–C = O), 21.98 (CO–*CH*$_3$), 21.38 (–*CH*$_2$–CH_3), 21.07 (*CH*$_3$–CH–O), 19.64 (*CH*$_3$–CH–C=), 13.46 (–CH_2–*CH*$_3$); IR (neat) γ_{max} 3442, 2959, 2933, 2873, 1764, 1733, 1659, 1460, 1417, 1372, 1328, 1244, 1175, 1109, 1088, 1051, 1017, 981, 956, 855, 814, 775, 609 cm^{-1}; HRESIMS *m/z* (pos): 415.20879 $C_{22}H_{32}NaO_6$ (calcd. 415.20911).

*(1S,3S)-3-acetoxy-1-((3aR,7S,8aS)-7-methyl-3-methylene-2-oxo-3,3a,4,7,8,8a-hexahydro-2H-cyclohepta[b]furan-6-yl)butyl octanoate (**5**)*

Yellowish gummy mass (This compound was prepared by reacting 100 mg isoxanthanol (**1**) with 90 µL octanoic anhydride and purified by the procedure as described above to obtained 137 mg with 98% yield); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 6.27$ (1H, d, $J = 3.2$ Hz, CH_2=C–), 5.81 (1H, dd, $J = 5.6$, 9.2 Hz,=CH–CH_2–), 5.52 (1H, d, $J = 2.8$ Hz, CH_2=C–), 5.08 (1H, dd, $J = 5.6$, 8.0 Hz, CH–$OCOCH_2$), 4.88 (1H, dt, $J = 6.4$, 12.4 Hz, CH–OAc), 4.63 (1H, ddd, $J = 2.8$, 8.8 Hz, CH–OCO), 3.27–3.43 (1H, m, CH–CH–), 2.58–2.65 (1H, m, CH_2–CH–), 2.44 (1H, m, CH_2–CH–), 2.27–2.93 (2H, m, CH–CH_2–), 2.06 (3H, s, CH_3–CO–), 2.01 (2H, m, –CH_2–CO–), 1.8 (2H, m, CH_2–CH_2–), 1.6 (2H, m, CH_2–CH_2–), 1.28–1.59 (9H, m, –CH_2–CH_2–CH_2–CH_2–CH–), 1.26 (3H, d, $J = 6.4$ Hz, CH_3–), 1.11 (3H, d, $J = 7.5$ Hz, CH_3–CH–), 0.87 (3H, q, $J = 6.8$ Hz, CH_3–CH_2). ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta =$ 173.17 (*C*=O), 170.41 (*C*=O), 170.06 (*C*=O), 144.43 (*C*=CH), 138.59 (CH_2=*C*), 124.76 (*CH*$_2$=C), 122.23 (*CH*=C), 78.83 (*CH*–O), 74.60 (*CH*–OAc), 67.93 (*CH*–OH), 41.34 (O–CH–*CH*$_2$–CH–O), 39.78 (CH_2–*CH*–CH–O), 36.89 (CH–*CH*$_2$–CH–O), 34.54 (CH_2–*CH*$_2$–C=O), 33.70 (=CH–*CH*$_2$–CH), 31.69 (–*CH*$_2$–CH_2–), 29.10 (–*CH*$_2$–CH_2–), 28.92 (–*CH*$_2$–CH_2–), 26.15 (=C–*CH*–CH_3), 24.90 (–*CH*$_2$–CH_2–C=O), 22.59 (CO–*CH*$_3$), 21.61 (*CH*$_3$–CH–O), 21.32 (–*CH*$_2$–CH_3), 19.86 (*CH*$_3$–CH–C=), 14.07 (–CH_2–*CH*$_3$); IR (neat) γ_{max} 3437, 2955, 2928, 2856, 1765, 1735, 1656, 1459, 1373, 1327, 1243, 1159, 1115, 1051, 1018, 957, 855, 813, 773, 609,

515 cm^{-1}; HRESIMS *m/z* (pos): 457.2556 for $C_{25}H_{38}NaO_6$ (calcd 457.2560).

*(1S,3S)-3-acetoxy-1-((3aR,7S,8aS)-7-methyl-3-methylene-2-oxo-3,3a,4,7,8,8a-hexahydro-2H-cyclohepta[b]furan-6-yl)butyl decanoate (**6**)*

Yellowish gummy mass (This compound was prepared by reacting 100 mg isoxanthanol (**1**) with 90 µL decanoic anhydride and purified by the procedure as described above to obtained 147 mg with 98% yield); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 6.26$ (1H, d, $J = 3.21$ Hz, CH_2=C–), 5.81 (1H, dd, $J = 5.6$, 9.2 Hz,=CH–CH_2–), 5.52 (1H, d, $J = 2.8$ Hz, CH_2=C–), 5.08 (1H, dd, $J = 5.6$, 8.4 Hz, –CH–$OCOCH_2$), 4.87 (1H, dt, $J = 6.0$, 12.4 Hz, CH–OAc), 4.62 (1H, m, CH–OCO–), 3.25–3.38 (1H, m, CH–CH–), 2.51–2.65 (1H, m, CH_2–CH–), 2.40–2.50 (1H, m, CH_2–CH–), 2.20–2.30 (2H, m, –CH_2–CH–), 2.03 (3H, s, CH_3–CO–), 1.81–1.89 (1H, m, CH_2–CH_2–), 1.72–1.80 (2H, m, –CH–CH_2–), 1.52–1.62 (2H, m, CH_2–CH_2–), 1.26 (17H, m, –$(CH_2)_7$– and CH_3–CH–), 1.13 (3H, d, $J = 6.90$ Hz, CH_3–CH–), 0.87 (3H, q, $J = 7.0$ Hz, CH_3–CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 173.11$ (<u>C</u>=O), 170.34 (<u>C</u>=O), 169.96 (<u>C</u>=O), 144.49 (<u>C</u>=CH), 138.65 (CH_2=<u>C</u>), 124.74 (<u>CH_2</u>=C), 122.10 (<u>CH</u>=C), 78.78 (<u>CH</u>–O), 74.58 (<u>CH</u>–OAc), 67.93 (<u>CH</u>–OH), 41.37 (O–CH–<u>CH_2</u>–CH–O), 39.83 (CH_2–<u>CH</u>–CH–O), 36.91 (CH–<u>CH_2</u>–CH–O), 34.55 (CH_2–<u>CH_2</u>–C=O), 33.70 (=CH–<u>CH_2</u>–CH), 31.83 (–<u>CH_2</u>–CH_2–), 29.43 (–<u>CH_2</u>–CH_2–), 29.24 (–<u>CH_2</u>–CH_2–), 29.14 (2 × –<u>CH_2</u>–CH_2), 26.18 (=C–<u>CH</u>–CH_3), 24.90 (–<u>CH_2</u>–CH_2–C=O), 22.63 (CO–<u>CH_3</u>), 21.58 (<u>CH_3</u>–CH–O), 21.27 (–<u>CH_2</u>–CH_3), 19.85 (<u>CH_3</u>–CH–C=), 14.06 (–CH_2–<u>CH_3</u>); IR (neat) γ_{max} 3436, 2926, 2854, 1766, 1735, 1657, 1459, 1372, 1326, 1243, 1157, 1115, 1050, 1017, 957, 855, 813, 773, 609, 521 cm^{-1}; HRESIMS *m/z* (pos): 485.2864 for $C_{27}H_{42}NaO_6$ (calcd 485.28736).

General procedures for preparation of aryl esters (7–10)

In a two-necked round bottom flask equipped with a calcium chloride drying tube was charged aryl carboxylic acid (1 equiv.), isoxanthanol (**1**) (1 equiv.), and DMAP (10 mol %) in dry dichloromethane. The resulting reaction mixture cooled in an ice bath to 0 °C and stirred for a while followed by addition of DCC (1.1 equiv.) at 0 °C over a period of 5 min. The ice bath was removed, and the dark-brown reaction mixture stirred for 3 h at room temperature. After completion of the reaction, the precipitate of dicyclohexylurea was removed by filtration through a fritted Buchner funnel, and the filtrate washed with water and extracted with dichloromethane. The organic layer was dried over anhydrous sodium sulfate and concentrated on a rotavapor. The crude product obtained was purified by CC on silica gel column using hexane-ethyl acetate as the eluent.

*(1S,3S)-3-acetoxy-1-((3aR,7S,8aS)-7-methyl-3-methylene-2-oxo-3,3a,4,7,8,8a-hexahydro-2H-cyclohepta[b]furan-6-yl)butyl 4-methyl-3,5-dinitrobenzoate (**7**)*

Yellowish powder (This compound was prepared by reacting 100 mg isoxanthanol (**1**) with 4-methyl-2,5-dinitro benzoic acid (73 mg) in presence of DCC (74 mg), DMAP (4 mg) by the procedure as described above to obtained 145 mg with 91% yield); mp 240–241 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = \delta$ 8.57 (2H, s, Ar–H), 6.27 (1H, d, $J = 3.21$ Hz, CH_2=C–), 5.95 (1H, dd, $J = 5.$ 54 and 9.2 Hz, =CH–CH_2–), 5.53 (1H, d, $J = 2.77$ Hz, CH_2=C–), 5.37 (1H, dd, $J = 5.81$, 8.32 Hz, CH–OCO–), 4.93 (1H, dt, $J = 6.0$, 12.4 Hz, CH–OAc), 4.64 (1H, ddd, $J = 2.8$, 8.8 Hz, CH–OCO–), 3.30–3.40 (1H, m, –CH_2–CH–), 2.70–2.75 (1H, m, CH_2–CH–), 2.64 (3H, s, Ar–CH_3), 2.25–2.29 (2H, m, CH–CH_2–), 2.06–2.13 (2H, m, CH_2–CH–), 2.02 (3H, s, CH_3–CO–), 1.89–2.0 (2H, m, CH_2–CH_2–), 1.29 (3H, d, $J = 6.28$ Hz, CH_3–CH–), 1.17 (3H, d, $J = 6.9$ Hz, CH_3–CH–); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 170.78$ (<u>C</u>=O), 170.32 (<u>C</u>=O), 162.50 (Ar–<u>C</u>=O), 151.80 (2 × Ar–<u>C</u>–NO_2), 143.36 (<u>C</u>=CH), 138.46 (CH_2=<u>C</u>), 131.77 (Ar–<u>C</u>–CH_3), 130.39 (Ar–<u>C</u>–), 127.78 (2 × Ar–<u>CH</u>), 126.65 (<u>CH_2</u>=C), 122.40 (<u>CH</u>=C), 78.59 (<u>CH</u>–O), 77.87 (<u>CH</u>–OAc), 67.77 (<u>CH</u>–OH), 41.29 (O–CH–<u>CH_2</u>–CH–O), 39.93 (CH_2–<u>CH</u>–CH–O), 36.84 (CH–<u>CH_2</u>–CH–O), 33.70 (=CH–<u>CH_2</u>–CH), 26.36 (=C–<u>CH</u>–CH_3), 21.74 (CO–<u>CH_3</u>), 21.25 (<u>CH_3</u>–CH–O), 21.14 (<u>CH_3</u>–CH–C=), 15.24 (Ar–<u>CH_3</u>); IR (neat) γ_{max} 3436, 3091, 2927, 2853, 1762, 1729, 1624, 1542, 1458, 1347, 1276, 1243, 1204, 1158, 1123, 1088, 1050, 1018, 956, 904, 857, 814, 744, 722, 609 cm^{-1}; HRESIMS *m/z* (pos): 539.16198 $C_{25}H_{28}N_2NaO_{10}$ (calcd. 539.16361).

*(1S,3S)-3-acetoxy-1-((3aR,7S,8aS)-7-methyl-3-methylene-2-oxo-3,3a,4,7,8,8a-hexahydro-2H-cyclohepta[b]furan-6-yl)butyl 2-(naphthalen-1-yl)acetate (**8**)*

Colorless liquid (This compound was prepared by reacting 100 mg isoxanthanol (**1**) with naphthyl acetic acid (60 mg) in presence of DCC (74 mg), DMAP (4 mg) by the procedure as described above to obtained 149 mg with 97% yield); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 8.04$ (1H, d, $J =$ 8.31 Hz, Ar–H), 7.85 (1H, d, $J = 7.82$ Hz, Ar–H), 7.75 (1H, d, $J = 7.84$ Hz, Ar–H), 7.52 (1H, d, $J = 1.22$ Hz, Ar–H) 7.47–7.50 (3H, m, Ar–H) 6.18 (1H, d, $J = 3.2$ Hz, CH_2=C–), 5.42 (1H, dd, $J = 5.6$, 9.2 Hz, =CH–CH_2–), 5.37(1H, d, $J = 3.63$ Hz, CH_2=C–), 4.98 (1H, dd, $J = 5.6$, 8.4 Hz, –CH–$OCOCH_2$), 4.88 (1H, dt, $J = 6.0$, 12.4 Hz, CH–OAc), 4.17 (1H, ddd, $J = 2.8$, 8.8 Hz, CH–OCO–),

4.04 (2H, s, Ar–CH_2–CO–), 2.51–2.65 (1H, m, CH_2–CH–), 2.34–2.39 (1H, m, CH_2–CH–), 2.11–2.23 (1H, m, CH–CH_2–), 2.0–2.02 (1H, m, CH_2–) 2.01(3H, s, CH_3–CO–), 1.70–1.80 (2H, m, CH_2–CH_2–), 1.60–1.70 (2H, m, CH_2–CH_2–), 1.19 (3H, d, $J = 6.27$ Hz, CH_3–CH–), 0.92 (3H, d, $J = 6.9$ Hz, CH_3–CH–); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 170.80$ (Ar–CH_2<u>C</u>=O), 170.37 (<u>C</u>=O), 170.00 (<u>C</u>=O), 144.50 (<u>C</u>=CH), 138.68 (CH_2=<u>C</u>), 133.81 (Ar–<u>C</u>–), 131.94 (Ar–<u>C</u>–), 130.32 (Ar–<u>CH</u>), 128.74 (Ar–<u>CH</u>), 128.32 (Ar–<u>CH</u>), 128.09 (Ar–<u>CH</u>), 126.31 (<u>CH_2</u>=C), 125.89 (Ar–<u>CH</u>), 125.57 (Ar–<u>C</u>–), 124.11 (Ar–<u>CH</u>), 123.90 (Ar–<u>CH</u>), 121.93 (<u>CH</u>=C), 78.66 (<u>CH</u>–O), 74.63 (<u>CH</u>–OAc), 68.00 (<u>CH</u>–OH), 40.92 (O–CH–<u>CH_2</u>–CH–O), 39.89 (CH_2–<u>CH</u>–CH–O), 39.75 (Ar–<u>CH_2</u>–), 36.56 (CH–<u>CH_2</u>–CH–O), 34.46 (=CH–<u>CH_2</u>–CH), 25.99 (=C–<u>CH</u>–CH_3), 21.30 (CO–<u>CH_3</u>), 21.15 (<u>CH_3</u>–CH–O), 19.63 (<u>CH_3</u>–CH–C=); IR (neat) γ_{max} 3442, 2956, 2926, 2871, 1762, 1732, 1658, 1597, 1511, 1459, 1372, 1327, 1245, 1128, 1088, 1050, 1018, 956, 856, 783, 609, 539, 510 cm^{-1}; HRESIMS *m/z* (pos): 499.2091 $C_{29}H_{32}NaO_6$ (calcd. 499.20911).

*(1S,3S)-3-acetoxy-1-((3aR,7S,8aS)-7-methyl-3-methylene-2-oxo-3,3a,4,7,8,8a-hexahydro-2H-cyclohepta[b]furan-6-yl)butyl 5-nitrofuran-2-carboxylate (**9**)*

Yellowish liquid (This compound was prepared by reacting 100 mg isoxanthanol (**1**) with nitro furanoic acid (51 mg) in presence of DCC (74 mg), DMAP (4 mg) by the procedure as described above to obtained 140 mg with 97% yield); ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 7.35$ (1H, d, $J = 3.7$ Hz, Ar–H), 7.30 (1H, d, $J = 3.80$ Hz), 6.27 (1H, d, $J = 3.3$ Hz, CH_2=C–), 5.94 (1H, dd, $J = 5.54$ and 9.2 Hz,=CH–CH_2–), 5.53 (1H, d, $J = 2.8$ Hz, CH_2=C–), 5.34 (1H, dd, $J = 5.6$, 8.4 Hz, CH–OCO–Ar), 4.96 (1H, dt, $J = 6.0$, 12.4 Hz, CH–OAc), 4.65 (1H, ddd, $J = 2.8$, 8.8 Hz, CH–OCO–), 3.27–3.32 (1H, m, –CH_2–CH–), 2.60–2.70 (1H, m, CH_2–CH–), 2.40–2.51 (1H, m, CH_2–CH–), 2.15–2.33 (1H, m, CH–CH_2–), 2.0–2.13 (2H, m, CH_2–CH–), 2.03 (3H, s, CH_3–CO–), 1.83–1.91 (2H, m, CH_2–CH_2–), 1.19 (3H, d, $J = 6.89$ Hz, CH_3–CH–), 0.86 (3H, d, $J = 6.9$ Hz, CH_3–CH–); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 170.39$ (<u>C</u>=O), 169.91 (<u>C</u>=O), 168.71 (<u>C</u>=O), 163.12 (Ar–<u>C</u>–NO_2), 144.71 (Ar–<u>C</u>–), 143.17 (<u>C</u>=CH), 138.48 (CH_2=<u>C</u>), 126.76 (<u>CH_2</u>=C), 122.36 (<u>CH</u>=C), 119.05 (Ar–<u>CH</u>), 111.45 (Ar–<u>CH</u>), 78.62 (<u>CH</u>–O), 77.81 (<u>CH</u>–OAc), 67.72 (<u>CH</u>–OH), 41.24 (O–CH–<u>CH_2</u>–CH–O), 39.67 (CH_2–<u>CH</u>–CH–O), 36.88 (CH–<u>CH_2</u>–CH–O), 33.5 2(=CH–<u>CH_2</u>–CH), 26.30 (=C–<u>CH</u>–CH_3), 21.68 (CO–<u>CH_3</u>), 21.25 (<u>CH_3</u>–CH–O), 20.12 (<u>CH_3</u>–CH–C= IR (neat) γ_{max} 3400, 2923, 2851, 1761, 1734, 1625, 1582, 1539, 1351, 1275, 1244, 1190, 1127, 1019, 959, 812, 765, 610 cm^{-1}; HRESIMS *m/z* (pos): 470.14148 $C_{22}H_{25}NNaO_9$ (calcd. 470.14152).

*(E)-(1S,3S)-3-acetoxy-1-((3aR,7S,8aS)-7-methyl-3-methylene-2-oxo-3,3a,4,7,8,8a-hexahydro-2H-cyclohepta[b]furan-6-yl)butyl 3-(4-methoxyphenyl)acrylate (**10**)*

Yellowish liquid (This compound was prepared by reacting 100 mg isoxanthanol (**1**) with 4-methoxycinnamic acid (57 mg) in presence of DCC (74 mg), DMAP (4 mg) by the procedure as described above to obtained 150 mg with 99% yield); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.98$ (1H, d, $J =$ 16.14 Hz, –CH=CH–), 7.50 (1H, m, Ar–H), 7.35 (1H, m, Ar–H), 6.94 (2H, m, Ar–H), 6.49 (1H, d, $J = 16.14$ Hz, –CH=CH–), 6.26 (1H, d, $J = 3.2$ Hz, CH_2=C–), 5.87 (1H, dd, $J = 5.54$, 9.2 Hz,=CH–CH_2–), 5.52 (1H, d, $J = 2.8$ Hz, CH_2=C–), 5.20 (1H, dd, $J = 5.6$, 8.4 Hz, CH–OCO–CH=), 4.96 (1H, dt, $J = 6.0$ and 12.4 Hz, CH–OAc), 4.66 (1H, ddd, $J = 2.8$, 8.8 Hz, CH–OCO–), 3.89 (3H, s, Ar–OCH_3), 3.31 (1H, m, –CH–CH_2–), 2.52–2.67 (1H, m, CH_2–CH–), 2.32–2.51 (1H, m, CH_2–CH–), 2.22–2.31 (1H, m, CH–CH_2–), 2.1–2.20 (1H, m, CH–CH_2–), 2.01 (3H, s, CH_3–CO–), 1.86–2.05 (1H, m, CH_2–CH–) 1.79–1.83 (2H, m, CH_2–CH_2–), 1.25 (3H, d, $J = 6.3$ Hz, CH_3–CH–), 1.20 (3H, d, $J = 6.9$ Hz, CH_3–CH–); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 170.41$ (<u>C</u>=O), 170.05 (<u>C</u>=O), 166.75 (CH=CH<u>C</u>=O), 158.43 (Ar–<u>C</u>–O–), 144.62 (<u>C</u>=CH), 140.78 (Ar–<u>CH</u>=CH–), 138.72 (CH_2=<u>C</u>), 131.68 (Ar–<u>CH</u>), 129.03 (Ar–<u>C</u>–), 124.59 (<u>CH_2</u>=C), 123.23 (Ar–<u>CH</u>), 122.06 (Ar–<u>CH</u>), 120.73 (Ar–<u>CH</u>), 118.34 (Ar–CH=<u>CH</u>–), 111.20 (Ar–<u>CH</u>), 78.87 (<u>CH</u>–O), 74.73 (<u>CH</u>–OAc), 68.15 (<u>CH</u>–OH), 55.51 (<u>CH_3</u>–O–), 41.37 (O–CH–<u>CH_2</u>–CH–O), 39.96 (CH_2–<u>CH</u>–CH–O), 36.95 (CH–<u>CH_2</u>–CH–O), 33.70 (=CH–<u>CH_2</u>–CH), 26.21 (=C–<u>CH</u>–CH_3), 21.63 (CO–<u>CH_3</u>), 21.30 (<u>CH_3</u>–CH–O), 19.91 (<u>CH_3</u>–CH–C=); IR (neat) γ_{max} 3400, 2929, 2873, 2849, 1762, 1734, 1707, 1628, 1597, 1576, 1488, 1464, 1437, 1372, 1322, 1246, 1161, 1122, 1087, 1049, 956, 869, 814, 757, 609 cm^{-1}; HRESIMS *m/z* (pos): 491.20417 $C_{27}H_{32}NaO_7$ (calcd. 491.20402).

General procedure for preparation of amine adducts (11–12) of isoxanthanol

The amine adducts were prepared using 1 equiv. of isoxanthanol (**1**) with 2 equiv. of respective amine in methanol and the resulting reaction mixture stirred at room temperature for 6 h. The reaction monitored by TLC, and after completion of the reaction, the contents poured in water, extracted with ethyl acetate and concentrated on thin film evaporator under reduced pressure to give the crude product which on CC in mobile phase 20% EtoAc in hexane afforded the amine adducts **11** and **12**.

*(2S,4S)-4-((3S,3aR,7S,8aS)-3-((dimethylamino)methyl)-7-methyl-2-oxo-3,3a,4,7,8,8a-hexahydro-2H-cyclohepta[b]furan-6-yl)-4-hydroxybutan-2-yl acetate (**11**)*

Yellowish liquid (This compound was prepared by reacting 100 mg isoxanthanol (**1**) with dimethylamine (140 mg) to obtained 103 mg with 91% yield); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 5.69$ (1H, dd, $J = 5.53$, 9.1 Hz,=CH–CH_2–), 4.97 (1H, dt, $J = 6.0$, 12.4 Hz, CH–OAc), 4.48 (1H, ddd, $J = 2.8$, 8.8 Hz, CH–OCO–), 4.08 (1H, dd, $J = 5.6$, 8.4 Hz, CHOH), 2.65 (5H, m, –NCH_3, –NCH_2), 2.42 (4H, m, –NCH_3, –CH–CH_2–), 2.24 (6H, m,–CH_2–, –CH_2–CH–, –CH_2–CH–), 2.04 (3H, s, CH_3CO–), 1.89 (2H, m, –CH–CH–), 1.67 (3H, d, $J = 6.6$ Hz, CH_3–CH–), 1.23 (3H, d, $J = 6.9$ Hz, CH_3–CH–); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 177.60$ (*C*=O), 170.65 (*C*=O), 138.58 (CH=*C*), 124.01 (*CH*=C), 78.95 (*CH*–O), 73.59 (*CH*–OAc), 69.31 (*CH*–OH), 65.80 (–N–CH_2), 59.24 (CH_2–*CH*–CH–O), 45.60 (–CH–*CH*–O), 43.43 (O–CH–CH_2–CH–O), 35.85 (CH–CH_2–CH–O), 32.85 (=CH–CH_2–CH), 26.01 (=C–*CH*–CH_3), 23.63 (–N–CH_3), 22.07 (CO–CH_3), 21.40 (CH_3–CH–O), 20.36 (CH_3–CH–C=); IR (neat) γ_{max} 3443, 2921, 1619, 1415, 1020, 773, 607, 519, 506 cm^{-1}; HRESIMS *m/z* (pos): 354.2269 $C_{19}H_{32}NO_5$ (calcd. 354.2275).

*(2S,4S)-4-hydroxy-4-((3S,3aR,7S,8aS)-7-methyl-3-((4-methylpiperazin-1-yl)methyl)-2-oxo-3,3a,4,7,8,8a-hexahydro-2H-cyclohepta[b]furan-6-yl)butan-2-yl acetate (**12**)*

Yellowish liquid (This compound was prepared by reacting 100 mg isoxanthanol (**1**) with *N*-methyl piperzine (32 mg) to obtained 100 mg with 89% yield); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 5.67$ (1H, dd, $J = 5.51$, 9.0 Hz, =CH–CH_2–), 4.94 (1H, dt, $J = 6.03$, 12.29 Hz, CH–OAc), 4.44 (1H, ddd, $J = 3.0$, 8.8 Hz, CH–OCO–), 4.05 (1H, dd, $J = 5.7$, 8.6 Hz, CHOH), 3.36 (4H, m, –$N(CH_2)_2$), 2.63 (4H, m, –$N(CH_2)_2$), 2.47 (5H, m, –NCH_2, –CH_2– and –CH–), 2.42 (4H, –CH_2– and –CH_2–), 2.32 (3H, *s*, –NCH_3), 2.04 (3H, s, CH_3CO–), 1.89 (2H, m, –CH_2), 1.67 (3H, d, $J = 6.59$ Hz, CH_3–CH–), 1.23 (3H, d, $J = 6.80$ Hz, CH_3–CH–); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 177.81$ (*C*=O), 170.65 (*C*=O), 144.27 (CH=*C*), 124.69 (*CH*=C), 79.24 (*CH*–O), 73.42 (*CH*–OAc), 69.36 (*CH*–OH), 65.72 (–N–CH_3), 58.04 (–N–CH_2), 57.75(–N–CH_2), 54.77 (–N–CH_2), 45.57 (–CH–*CH*–O), 43.29 (O–CH–CH_2–CH–O), 35.85 (CH–CH_2–CH–O), 32.53 (=CH–CH_2–CH), 26.63 (=C–*CH*–CH_3), 23.71 (CH_2–*CH*–CH–O), 21.86 (CO–CH_3), 21.44 (CH_3–CH–O), 20.31 (CH_3–CH–C=); IR (neat) γ_{max} 3436, 2921, 2851, 1733, 1619, 1374, 1247, 1020, 771, 607, 522, 503 cm^{-1}. HRESIMS *m/z* (pos): 409.2696 $C_{22}H_{37}N_2O_5$ (calcd. 409.2624).

*(2S,4S)-4-hydroxy-4-((3R,3aR,7S,8aS)-7-methyl-2-oxo-2,3a,4,4′,5′,7,8,8a-octahydrospiro[cyclohepta[b]furan-3,3′-pyrazol]-6-yl)butan-2-yl acetate (**13**)*

Yellowish liquid (This compound was prepared by reacting 100 mg isoxanthanol (**1**) with diazomethane in ether at 0 °C to obtained 111 mg with 98% yield); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 5.66$ (1H, dd, $J = 5.5$, 8.9 Hz, =CH–CH_2–), 4.95 (1H, dt, $J = 6.02$, 12.27 Hz, CH–OAc), 4.46 (1H, ddd, $J = 3.01$, 8.78 Hz, CH–OCO–), 4.07 (1H, dd, $J = 5.67$, 8.72 Hz, –CHOH), 3.49 (2H, m, –CH_2–N=), 2.30 (5H, m, –CH_2–, –CH_2– and –CH–), 2.3 (3H, m, –CH_2– and –CH–), 2.04 (3H, s, CH_3CO–), 1.88 (2H, m, –CH_2–), 1.65 (3H, d, $J = 6.59$ Hz, CH_3–CH–), 1.21 (3H, d, $J = 6.80$ Hz, CH_3–CH–). ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 177.47$ (*C*=O), 170.76 (*C*=O), 141.04 (–CH=*C*–), 131.98 (–*CH*=C–), 96.72 (–*C*–COO), 76.13 (*CH*–O), 75.00 (=NCH_2–), 72.86 (*CH*–OAc), 70.09 (*CH*–OH), 51.12 (–*CH*–CH–O), 44.57 (–CH_2–C–), 44.01 (O–CH–CH_2–CH–O), 32.60 (=CH–CH_2–CH), 30.49 (–CH–CH_2–CH–O), 29.97 (CH_3–*CH*–), 21.84 (CO–CH_3), 21.32 (CH_3–CH–O), 21.19 (CH_3–CH–C=); HRESIMS *m/z* (pos): 351.19144 $C_{18}H_{27}N_2O_5$ (calcd. 351.1842).

*(3aR,7S,8aS)-6-((1S,3S)-1,3-dihydroxybutyl)-7-methyl-3-methylene-3,3a,4,7,8,8a-hexahydro-2H-cyclohepta[b]furan-2-one (**14**)*

Yellowish liquid (This compound was prepared by enzymatic deacetylation of 100 mg isoxanthanol (**1**) using Lipase AS Amano (1 mg) in water to obtained 84 mg with 98% yield); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 6.26$ (1H, d, $J = 3.22$ Hz, CH_2=C–), 5.81 (1H, dd, $J = 5.64$, 9.21 Hz, =C*H*–CH_2–), 5.54 (1H, d, $J = 2.83$ Hz, –CH_2=C–), 4.63 (1H, dt, $J = 6.03$, 12.42 Hz, –C*H*OCO–), 4.08 (1H, dd, $J = 5.63$, 8.42 Hz, C*H*OH) 3.34 (1H, m, –C*H*CH–), 2.63 (1H, m, –CH_2CH–), 2.47 (1H, m, CH_2–CH–), 2.32 (1H, m, C*H*–CH_2–), 1.82 (1H, m, C*H*–CH_2–), 1.73 (2H, m, CH–CH_2–), 1.60 (2H, m, CH_2–CH_2–), 1.24 (3H, d, $J = 6.46$ Hz, CH_3–CH–), 1.19 (3H, d, $J = 6.83$ Hz, CH_3–CH–); ^{13}C NMR ($CDCl_3$, 125 MHz): $\delta = 170.54$ (*C*=O), 148.32 (CH=*C*), 138.65 (CH_2=*C*), 122.30 (*CH*$_2$=C), 121.57 (*CH*=C), 79.29 (*CH*–O), 76.24 (CH_3–*CH*–OH), 68.84 (–CH_2*CH*–OH), 44.05 (O–CH–CH_2–CH–O), 41.50 (CH_2–*CH*–CH–O), 36.81 (CH–CH_2–CH–O), 33.28 (=CH–CH_2–CH), 25.95 (=C–*CH*–CH_3), 24.16 (CH_3–CH–O), 21.86 (CH_3–CH); IR (neat) γ_{max} 3446, 2921, 2851, 1732, 1620, 1374, 1246, 1019, 607, 503 cm^{-1}; HRESIMS *m/z* (pos): 289.1408 $C_{15}H_{22}NaO_4$ (calcd. 289.159).

Biological assays

SRB assay for growth inhibition

SRB is a bright-pink aminoxanthene dye that binds to basic amino acid residues present on TCA fixed cells. Under mild acidic conditions it easily binds to cells and can be extracted under mild basic conditions. The amount of dye extracted is directly proportional to the cell mass. Briefly, cell suspensions of optimum density (7000–12,000 cells/100 µL) were seeded and incubated for 24 h. Test compounds in complete growth medium were added along with Erlotinib and Paclitaxel as positive controls. After 48 h incubation, cells were fixed with ice-cold TCA, washed, dried and stained with SRB dye (0.4% w/v SRB in 1% acetic acid for 30 min). The unbound SRB was removed by washing with 1% acetic acid and plates were dried. The bound dye was solubilized with 10 mM Tris buffer (pH = 10.4) and reading was taken at 540 nm on microplate reader (BioTek Synergy HT). For preliminary screening, 10 and 50 µM concentrations of test compounds were used whereas for IC_{50} calculation a range of concentrations were used. IC_{50} value was determined by non-linear regression analysis using Prism, version 5.04 from Graph Pad Software (La Jolla, CA) (Singh et al. 2013).

Cell cycle analysis by flow cytometry

A549 cells (1.2×105 cells/mL/six-well plate) were exposed to varying concentration of compound isoxanthanol cinnamate (**10**) for 24 h. Cells were then trypsinized, washed with PBS and fixed in 70% ethanol at 20 °C, overnight. After fixing, cells were again washed, digested with DNase free RNase (100 µg/mL) at 37 °C for 45 min and stained with propidium iodide (PI) (5 µg/mL). Cells were then analyzed on flow cytometer FACS Aria (Becton Dickinson, USA). Sub-G1 cell fraction represents apoptotic cell population (Majeed et al. 2013).

Measurement of mitochondrial membrane potential loss

Changes in mitochondrial transmembrane potential were measured after staining with rhodamine-123. A549 cells (1.2×105 cells/mL/six-well plate) were exposed to varying concentration of compound **10** for 24 h. Rh-123 (200 nM) was added to each well before 1 h termination. Cells were collected at 3000 rpm for 5 min, washed once with PBS, resuspended in 500 µL of PBS and mitochondrial membrane potential loss was analyzed on flow cytometer FACS Aria (Becton Dickinson, USA) (Singh et al. 2013).

Clonogenic assay

The clonogenic assay estimates the reproductive viability of cells treated with cytotoxic agents or anti-cancer therapeutics by assessing their colony formation ability. A549 cells were seeded in six-well tissue culture grade flasks at a seeding density of 1.2×10^5 cells/well. After 24 h cells were exposed to various concentrations of compound **10** and kept at 37 °C, 5% CO_2 and 98% RH. Paclitaxel was used as positive control. After 24 h treatment cells were harvested and counted. Equal numbers of cells were re-plated and kept in CO_2 incubator at 37 °C for a time equivalent to six cell divisions so that the untreated well contains sufficiently large clones of 50 or more cells. Medium was removed and colonies were gently rinsed with PBS. Cells were fixed with 4% paraformaldehyde for 30 min and then stained with 0.5% crystal violet solution for 1 h. Excess crystal violet was washed off with water and plates were dried. Colonies having 50 or more cells were counted (Bhushan et al. 2007).

Wound healing assay

Wound healing assay is used to study cell migration in vitro which mimics cell migration during wound healing in vivo. A wound is induced in cell monolayer and images captured at beginning and at closure of wound in untreated cells. A549 cells were seeded in 6-well tissue culture grade flasks at a seeding density of 8×10^5 cells/well and kept in CO_2 incubator for 24 h after which a confluent monolayer was obtained. Scrape-wound was induced using a 1000 µL sterile pipette tip. After removing the cellular debris with PBS, cells were exposed to various concentrations of compound **10** and kept in CO_2 incubator at 37 °C. Paclitaxel was used as positive control. At 0 h and 24 h treatment, wounded areas were photographed with (10× objective) Olympus 70× and percentage of wound closure was estimated (Franken et al. 2006).

Statistical analysis

Data expressed as mean SD or representative of one of three similar experiments unless otherwise indicated. Comparisons were made between control and treated groups using Student's *t*-test. $P > 0.05$ values were considered significant.

Results and discussion

Chemistry

Isoxanthanol (**1**) isolated from the *Xanthium spinosum* and well characterized through spectroscopic techniques and

data were found in agreement with literature. Further its absolute configuration was confirmed by X-ray crystallography (see Fig. 2) and this is the first report of the establishment of absolute stereochemistry by X-ray analysis. Isoxanthanol displayed anti-proliferative activity against different human cancer cell lines leading thereby to the establishment of its anticancer activity. Furthermore, chemical modification of the parent molecule was undertaken in a bid to generate more potent molecule/s than the parent compound **1**. The chemical modifications were carried out at different active sites of the molecule (Fig. 1) and that included (i) α-methylene part of γ-butyro lactone double bond (from the literature survey the exocyclic double bond seems to be as an important functionality for the chemical modification for anticancer activity) (Winters et al. 1969; Woods et al. 2013; Baraldi et al. 2004), (ii) on hydroxyl group, and (iii) acetyl group of the molecule.

Chemical modification at the hydroxyl group of compound **1** was carried out to get desired acyl, aryl, and cinnamyl ester derivatives **2–10** (Scheme 1). The formation of acyl derivatives were confirmed by IR, NMR, and mass spectral data, appearance of bands for carbonyl (–CO) group of ester moiety in IR spectrum at ~1733 cm^{-1}, and observance of signals for the protons of acetyl (CH_3CO), aryl (aromatic protons) and olefinic protons of the cinnamoyl moiety (–CH=CH–CO) in ^1H NMR spectrum. Further HRESIMS data corroborated with the assigned structure of the isoxanthanol derivatives.

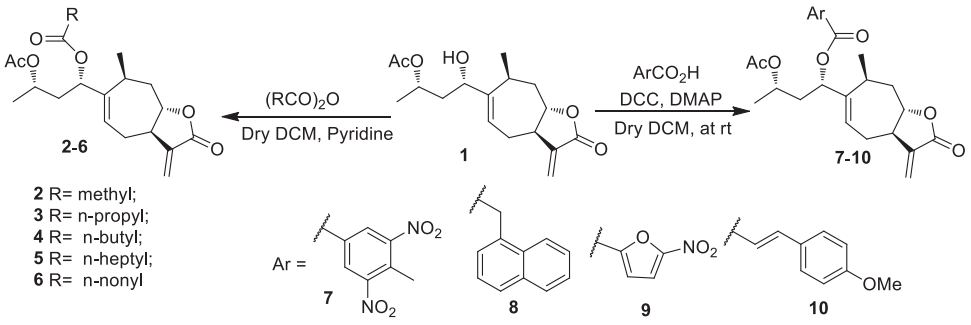
The chemical modification at the α-methylene group of the γ-butyro lactone of **1** was carried out to furnish amino adducts **11–12** (obtained through amination), and pyrazoline derivative **13** (obtained by diazomethane reaction) as shown in Scheme 2.

The chemical identity of the products was confirmed by spectral analysis i.e., appearance of additional signals of alkyl substituent of amine moiety of **11** and **12** in ^1H NMR and ^{13}C spectra along with disappearance of the signal for α-methylene (=CH_2) protons in **11**, **12**, and **13**, which was fully supported by HRMS data of the assigned chemical structures.

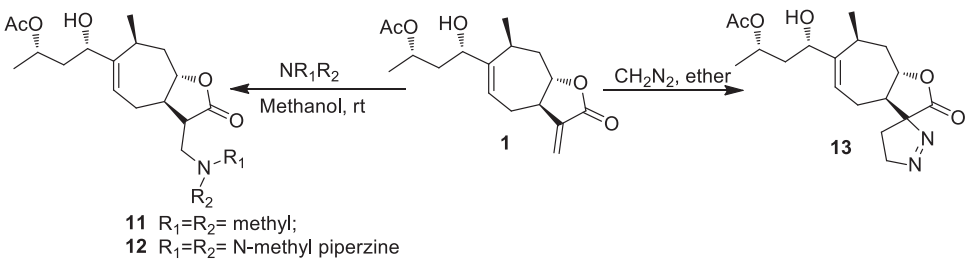
In an attempt for de-acetylation of **1** to obtain diol product (**14**) and bio-evaluate it vis a vis with the parent molecule for the activity profiling. Our initial attempts of de-acetylation through different reagents and conditions such as ammonia/methanol, K_2CO_3/methanol proved unsuccessful and complex mixture was obtained presuming involvement of nucleophilic addition as well as lactone ring opening reactions under the basic reaction conditions with perhaps solvent itself acting as a nucleophile. These complex reaction products were abandoned and not followed further. In order to get compound **14**, commercially available lipases were used and the reaction carried out under mild and neutral reaction conditions. The enzymatic reaction (Scheme 3) was successful and the desired product **14** was isolated in 98% yields (See Supporting Information, Table-S3).

Pharmacological evaluation

Isoxanthanol (**1**) and its derivatives (**2–14**) were screened (Table 1) at 10 and 50 µM concentration for their cytotoxic potential using sulforhodhamine B assay against four human cancer cell lines including lung (NCI-H322 and A549), glioblastoma (T98G) and epidermal (A431). Paclitaxel and erlotinib were taken as positive controls for the experiment. At the 50 µM concentration isoxanthanol derivatives **2–4**, **7**, **8**, **10**, and **11** showed significant activity



Scheme 1 Derivatization of isoxanthanol (**1**) at hydroxyl group



Scheme 2 Derivatization at α-methylene group of isoxanthanol (**1**)

Scheme 3 Enzyme catalyzed deacetylation of isoxanthanol (**1**)

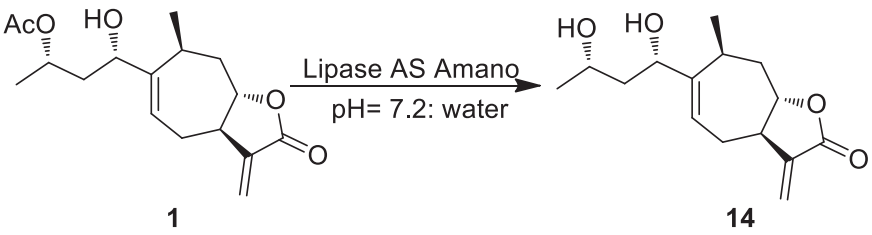


Table 1 In vitro screening of isoxanthanol (**1**) and its derivatives (**2–14**) against various human cancer cell lines

Compounds	Conc. (μM)	T98G	% Growth inhibition		
			A431	NCI-322	A549
1	10	0	6	11	9
	50	49	65	65	20
2	10	0	34	16	14
	50	**82**	**87**	60	**81**
3	10	17	54	8	43
	50	44	**100**	**100**	**91**
4	10	20	22	14	55
	50	**75**	**99**	**100**	**95**
5	10	0	4	9	42
	50	18	34	56	**89**
6	10	4	0	0	17
	50	22	14	9	**79**
7	10	0	0	13	6
	50	7	**100**	**80**	**90**
8	10	0	32	52	56
	50	**80**	**100**	**100**	**93**
9	10	0	0	3	0
	50	0	8	29	33
10	10	25	**99**	**89**	**95**
	50	**100**	**100**	**100**	**100**
11	10	0	21	0	35
	50	58	**93**	37	**95**
12	10	0	4	2	23
	50	9	58	16	50
13	10	0	14	13	8
	50	1	27	21	28
14	10	1	23	0	14
	50	4	44	32	38
Erlotinib	5	47	86	78	–
Paclitaxel	1	–	–	–	80

The bold values indicate growth inhibition more than 70%

against two or more human cancer cell lines (epidermal: A431, and lung: A549) than the parent molecule **1**. Compound **2** showed >80% cytotoxicity against T98G, A431 and A549 whereas **3**, **4**, and **7** (except NCI-H322 displaying 80% inhibition) showed >90% cytotoxicity against A431, NCI-H322 and A459 cell lines. Compound **8** displayed

Table 2 IC_{50} values (in μM) of selected compounds against human cancer cell lines

Tissue compounds cell line	Glioblastoma T98G	Epidermal A431	Lung NCI-H322	Lung A549
2	26.8 ± 2.8	10 ± 0.5	29 ± 5.7	18 ± 1.5
3	35 ± 3.6	6 ± 0.2	22 ± 1.7	7 ± 1.2
4	35.7 ± 1.7	12.2 ± 1.3	19.8 ± 2.2	7.2 ± 0.6
7	>50	21 ± 1	34 ± 4.5	17 ± 4.2
8	32 ± 1	9 ± 0.4	13 ± 0.5	5 ± 0.7
10	14 ± 1	2.3 ± 0.1	3 ± 0.2	1.9 ± 0.3
11	48.4 ± 4	16.4 ± 2	>50	14.3 ± 2

80% and >90% cytotoxicity against T98G and the rest of the cell lines respectively. Compound **11** exhibited >90% cytotoxicity against A431 and A549. Screening of the molecules at 10 μM concentration showed interesting results, in particular, for the compound **10** which displayed the best activity profile against A431, NCI-H322, and A549 cells lines and high percentage of growth inhibition (99, 89, and 95%) against the respective cell lines. The preliminary cytotoxic screening data of these compounds at the 10 and 50 μM is shown in Table 1. The compounds that were showed more potent activity than isoxanthanol were further selected for the determination of their IC_{50} values (Table 2) by non-linear regression analysis using Prism, version 5.04 from Graph Pad Software (La Jolla, CA).

Compounds **3**, **4**, **8**, and **10** displayed promising IC_{50} values of 7, 7.2, 5, and 1.9 μM respectively for the lung cancer line A549. Compounds **2**, **3**, **8**, and **10** displayed IC_{50} values of 10, 6, 9, and 2.3 μM for epidermal carcinoma A431. Since compound **10** exhibited maximum cytotoxic effect with IC_{50} values ranging from 1.9 to 14 μM against the four cell lines, it was, therefore, chosen for further mechanistic studies.

Cell death inducing potential of compound **10** was also assessed using flow cytometry through determination of sub-G1 cell population by PI staining. A549 cell line treated with **10** showed concentration dependent increase in apoptotic population after 24 h of incubation (Fig. 3) (Lowe and Lin 2000).

As compared to 3% in negative control, sub-G1 population was found 6.5, 11.3, 24.4, 38.8, and 51% at 2, 5, 10, 20, and 30 μM concentration of the compound **10**. In further

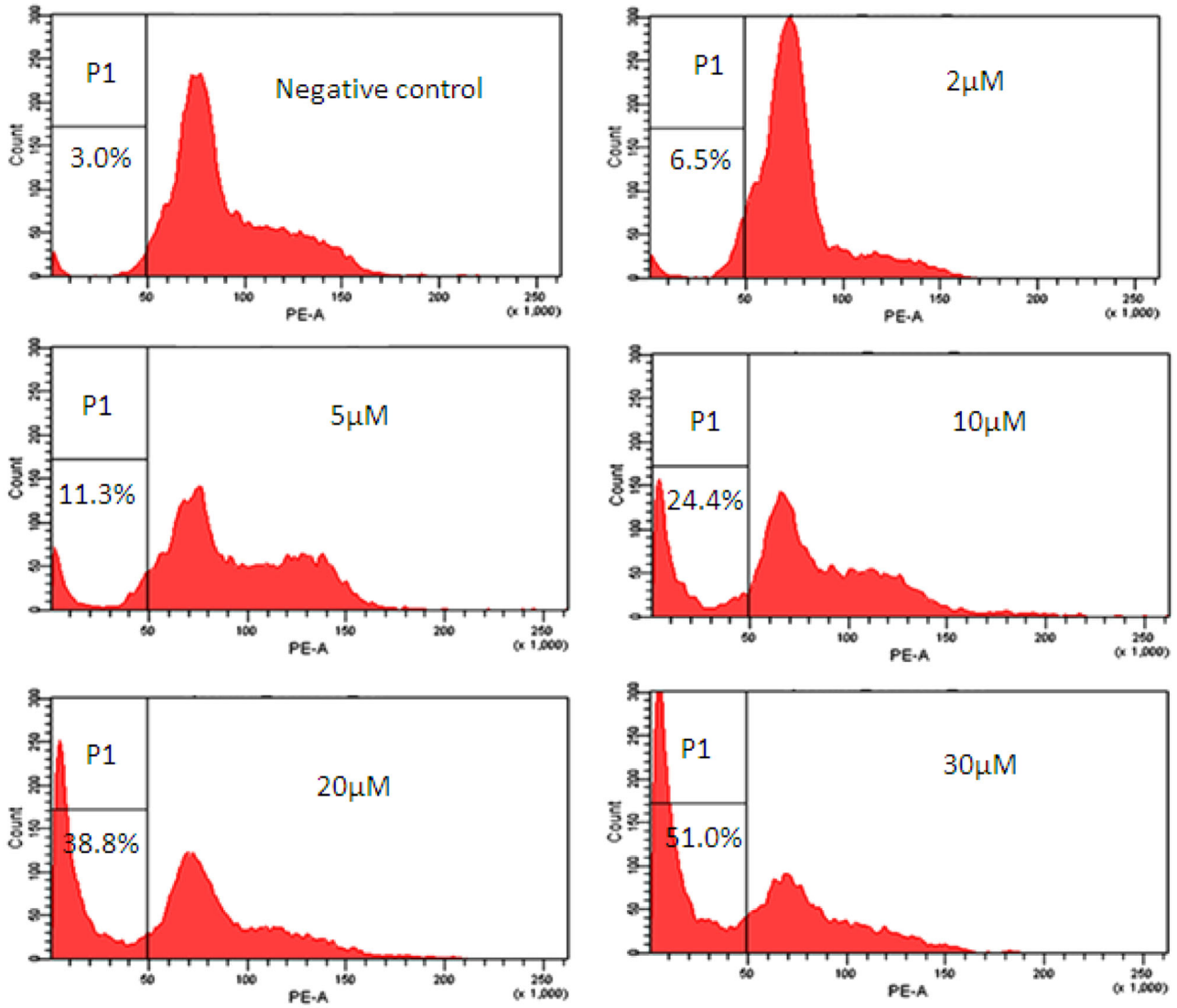


Fig. 3 Cell cycle analysis of compound **10** treated A549 cancer cells. A549 cells were treated with different conc. (2, 5, 10, 20, and 30 μM) of compound **10** to determine the sub-G1 (P1) population indicative of DNA damage

study, the effect of **10** on mitochondrial functioning was also investigated. Cytochrome-c is released on opening of mitochondrial permeability transition pore resulting in mitochondrial membrane potential loss (Heiden et al. 1997). A concentration dependent loss of mitochondrial membrane potential (Δψm) was established for compound **10** and same is shown in Fig. 4. While untreated control A549 cells showed 3.2% MMP loss, 5.1, 12.2, 46.5 65.3, and 96.7% MMP loss at 2, 5, 10, 20, and 30 μM concentration for compound **10**.

The clonogenic assay was also performed using A549 cells. Compound **10** produced concentration dependent inhibitory effect on the cells to reproduce and form colonies. The number of colonies formed at lower concentrations was comparable to untreated control but at higher concentration less number of colonies was formed. No colony was formed at 20 μM shown in Fig. 5.

Further, in vitro cell migration assay was performed to estimate the metastatic potential of A549 cells. Compound **10** treated cells were photographed and cell migration was assessed by comparing the gap size between the untreated and treated wells. A concentration dependent inhibitory effect could be seen on the motility of cells as shown in Fig. 6. The negative control had almost completely filled the scratched area, whereas treatment with 10 and 15 μM of compound **10** significantly ($p < 0.001$) inhibited motility of A549, as did treatment with paclitaxel.

With respect to structure–activity relationship, some trends could be observed. For example, influence of nature/size of the acyl substituent on the activity was observed, and

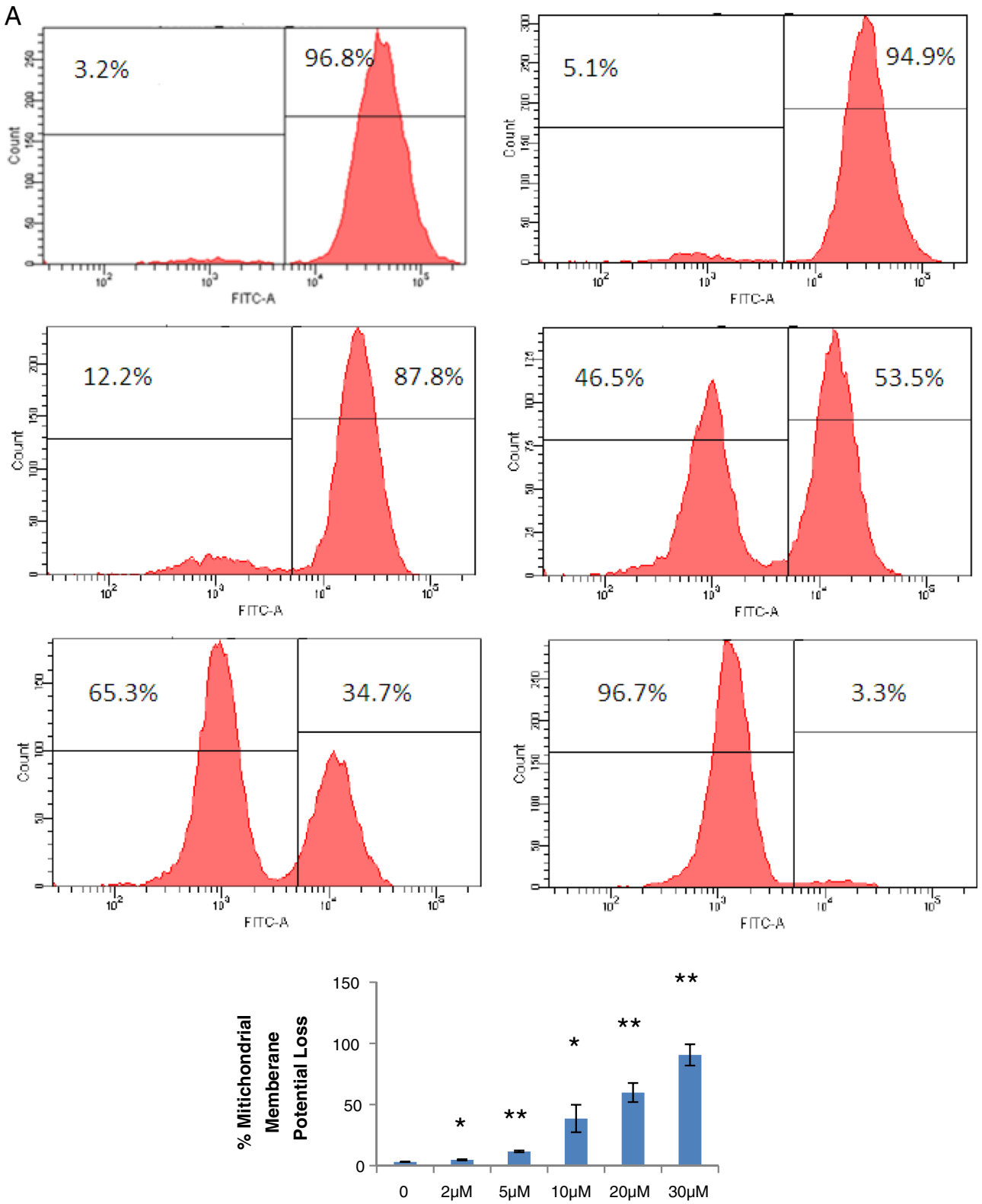


Fig. 4 **a** Compound **10** induced mitochondrial membrane potential loss in A549 cells. Cells were treated with different conc. (2, 5, 10, 20, and 30 μM) of **10** for 24 h. **b** Compound **10** induced mitochondrial membrane potential loss in A549 cells. Cells were treated with different conc. (2, 5, 10, 20, and 30 μM) of compound 10 for 24 h. Cells were stained with 200 nM Rh-123 and analyzed by flow cytometer

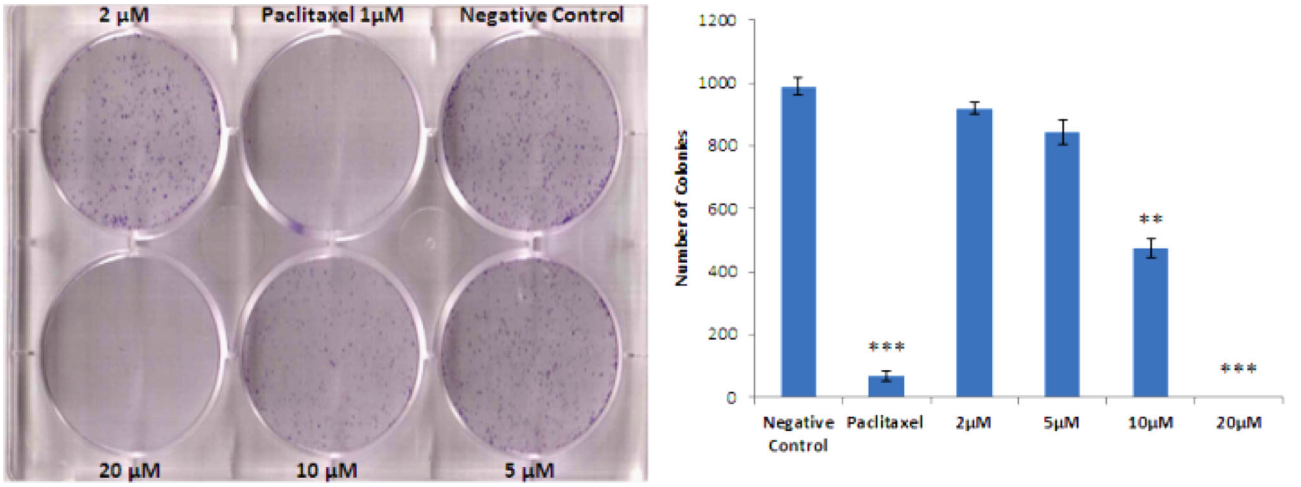


Fig. 5 Compound **10** inhibits the colony formation ability of A549 cells. Indicated concentrations of compound **10** were added to A549 cells. After 24 h treatment, cells were harvested; washed and equal number of cells from each treatment was re-seeded in six-well plate for a time equivalent to at least six cell divisions. Colonies were then fixed and stained with 0.5% crystal violet. Number of colonies was counted. $*P < 0.05$, $**P < 0.01$, $***P < 0.005$ Compared with untreated control

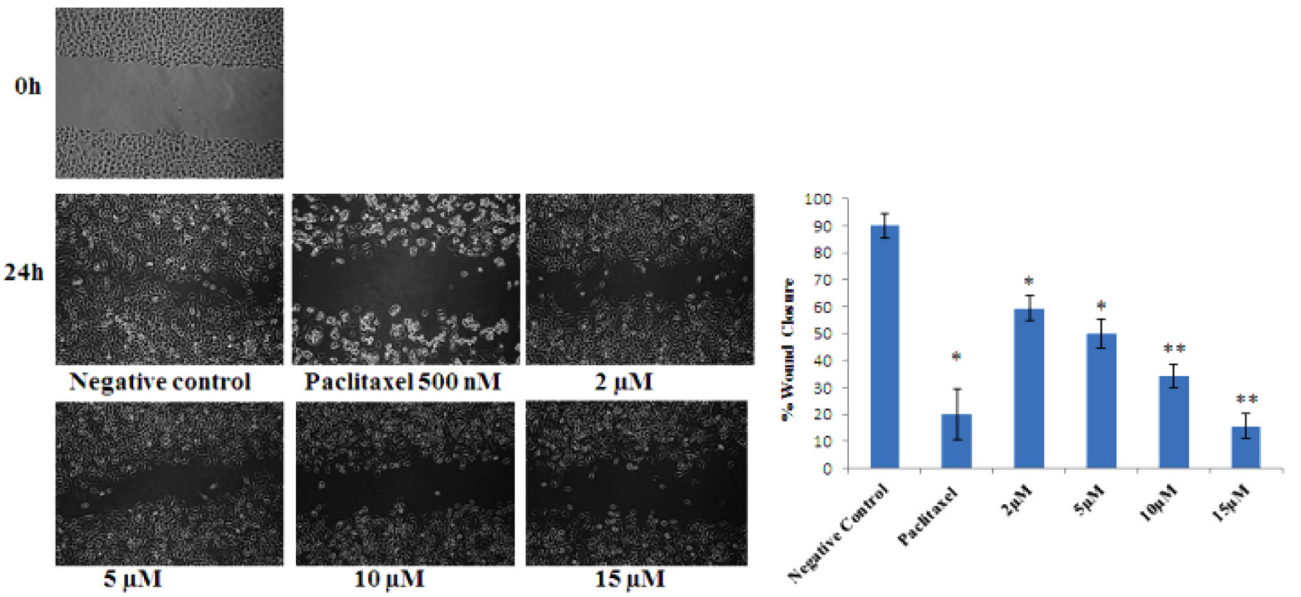


Fig. 6 Compound **10** inhibits the motility of A549 cells. A549 cells (8×105 cells/mL/six well plate) were grown to confluence and scratched with sterile tip: compound **10** was added as indicated. Scratched areas were photographed (Olympus 70×) at 0 h and then at 24 h to assess the degree of wound healing. Scratched areas were quantified in each treatment and data were calculated from two independent experiments. $*P < 0.05$, $**P < 0.001$ compared with untreated control

among the aliphatic acyl substituents, lower homologs exerted greater influence than the higher homologs resulting in the increase of activity. Among the aryl moiety bearing alkenoic substituents, electron releasing group showed augmentation in the activity of the compound and opposite effect was observed for the electron withdrawing groups. Loss of activity was observed for deacylated product showing thereby that acetyl group apparently contributes towards the anticancer activity of **1**. Similarly, loss of sp^2 character of the substituent in lactone ring also lead to deterrent effect on the activity.

Conclusion

In summary, the cytotoxic activity of sesquiterpene lactone isoxanthanol (**1**) and its thirteen analogs has been established against various human cancer cell lines and C-3

cinnamyl analog (**10**) found most active displaying single digit IC_{50} value for lung cancer cell line. Concentration dependent increase in apoptotic population after 24 h of incubation with 51% sub-G1population at 30 µM concentration against A549 cell line has been established for compound **10** (using flowcytometry). Colony formation on lung cancer cell line at 20 µM concentration has been established through clonogenic assay. In in vitro cell migration, the metastatic potential of A549 cells, has been arrested significantly ($p < 0.001$) at 10 and 15 µM concentration of potent molecule **10**.

Acknowledgements The CSIR is well acknowledged for financial support under 12th five-year project BSC0108. The authors thank Botany division of the Institute for providing the plant material, Instrumentation division for spectral data, and CSIR-UGC for the award of Fellowship to one of the author (PKC). IIIM publication no. IIIM/2011/2017.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Abdei-Mogib M, Dawidar AM, Metwally MA, Abou-Elzahab M (1991) Xanthanolides from *Xanthium spinosum*. Phytochemistry 30:3461–3462

Baraldi PG, Nunez MC, Tabrizi MA, Clercq ED, Balzarini J, Bermejo J, Estevez F, Romagnoli R (2004) Design, synthesis, and biological evaluation of hybrid molecules containing α-methylene-γ-butyrolactones and polypyrrole minor groove binders. J Med Chem 47:2877–2886

Bhushan S, Kumar A, Malik F, Andotra SS, Sethi VK, Kaur IP, Taneja SC, Qazi GN, Singh J (2007) A triterpenediol from *Boswellia serrata* induces apoptosis through both the intrinsic and extrinsic apoptotic pathways in human leukemia HL-60 cells. Apoptosis 12:1911–1926

Bohlmann F, Singh P, Joshi KC, Singh CL (1982) Xanthanolides from *Xanthium indicum*. Phytochemistry 21:1441–1443

Chinthakindi PK, Sangwan PL, Farooq S, Aleti RR, Kaul A, Saxena AK, Murthy YLN, Vishwakarma RA, Koul S (2013) Diminutive effect on T and B- cell proliferation of non-cytotoxic α-santonin derived 1,2,3-triazoles: a report. Eur J Med Chem 60:365–375

Chinthakindi PK, Singh J, Gupta A, Nargotra A, Mahajan P, Kaul A, Ahmed Z, Koul S, Sangwan PL (2017) Synthesis of α-santonin derivatives for diminutive effect on T and B-cell proliferation and their structure–activity relationship. Eur J Med Chem 127:1047–1058

Christensen SB, Skytte DM, Denmeade SR, Dionne C, Moller JV (2009) A Trojan horse in drug development: targeting of thapsigargins towards prostate cancer cells. Anticancer Agents Med Chem 9:276–294

Cragg GM, Grothaus PG, Newman DJ (2014) New horizons for old drugs and drug leads. J Nat Prod 77:703–723

Dangroo NA, Singh J, Gupta N, Singh SK, Koul A, Khuroo MA, Sangwan PL (2017) T- and B-cell immunosuppressive activity of novel α-santonin analogs with humoral and cellular immune response in mice. Med Chem Commun 8:211–219

Dar AA, Dangroo NA, Raina A, Qayum A, Singh SK, Kumar A, Sangwan PL (2016) Biologically active xanthones from *Codonopsis ovata*. Phytochemistry 132:102–108

Efferth T (2006) Molecular pharmacology and pharmacogenomics of artemisinin and its derivatives in cancer cell. Curr Drug Targets 7:407–421

Favier LF, Maria AOM, Wendel GH, Borkowski EJ, Giordano OS, Pelzer L, Tonn CE (2005) Anti-ulcerogenic activity of xanthanolide sesquiterpenes from *Xanthium cavanillesii* in rats. J Ethnopharmacol 100:260–267

Franken NAP, Rodermond HM, Stap J, Haveman J, Bree CV (2006) Clonogenic assay of cells in vitro. Nat Protoc 5:2315–2319

Gach K, Dalugosz A (2014) α-Methylene-γ-lactones as a novel class of anti-leukemic agents. Anticancer Agents Med Chem 14:688–694

Ghantous A, Gali-Muhtasib H, Vuorela H, Saliba NA, Darwichem N (2010) What made sesquiterpene lactones reach cancer clinical trials? Drug Discov Today 15:668–678

Ghantous A, Sinjab A, Herceg Z, Darwiche N (2013) Parthenolide: from plant shoots to cancer roots. Drug Discov Today 18:894–905

Gupta N, Sharma S, Raina A, Dangroo NA, Bhushan S, Sangwan PL (2016) Synthesis and anti-proliferative evaluation of novel 3,4-dihydro-2H-1,3-oxazine derivatives of bakuchiol. RSC Adv 6 (108):106150–106159

Harvey AL, Edrada-Ebe R, Quinn RJ (2015) The re-emergence of natural products for drug discovery in the genomics era. Nat Rev Drug Discov 14:111–129

Heiden MGV, Chandel NS, Williamson EK, Schumacker PT, Thompson CB (1997) Bcl-xL regulates the membrane potential and volume homeostasis of mitochondria. Cell 91:627–637

Jordan CT (2006) Searching for leukemia stem cells-not yet the end of the road? Cancer Cell 10:253–254

Khan I, Guru SK, Rath SK, Chinthakindi PK, Singh B, Koul S, Bhushan S, Sangwan PL (2016) A novel triazole derivative of betulinic acid induces extrinsic and intrinsic apoptosis in human leukemia HL-60 cells. Eur J Med Chem 108:104–116

Lowe SW, Lin AW (2000) Apoptosis in cancer. Carcinogenesis 21:485–495

Marco JA, Sanz-Cervera JF, Corral J, Carda M, Jakupovic J (1993) Xanthanolides from *Xanthium*: absolute configuration of xanthanol, isoxanthanol and their C-4 epimers. Phytochemistry 34:1569–1576

Majeed R, Reddy MV, Chinthakindi PK, Sangwan PL, Hamid A, Chashoo G, Saxena AK, Koul S (2012) Bakuchiol derivatives as novel and potent cytotoxic agents: a report. Eur J Med Chem 49:55–67

Majeed R, Sangwan PL, Chinthakindi PK, Dangroo NA, Thota N, Hamid A, Sharma PR, Saxena AK, Koul S (2013) Synthesis of 3-O-propargylated betulinic acid and its 1,2,3-triazoles as potential apoptotic agents. Eur J Med Chem 63:782–792

Newman DJ, Cragg GM (2012) Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 75:311–335

Nour AMM, Khalid SA, Kaiser M, Brun R, Abdallah WE, Schmidt TJ (2009) The antiprotozoal activity of sixteen asteraceae species native to sudan and bioactivity-guided isolation of xanthanolides from *Xanthium brasilicum*. Planta Med 75:1363–1368

Omar AA, Elrashidy EM, Ghazy NA, Metwally AM, Ziesche J, Bohlmann F (1984) Xanthanolides from *Xanthium spinosum*. Phytochemistry 23:915–916

Schmidt TJ (1997) Helenanolide-type sesquiterpene lactones-III Rates and stereochemistry in the reaction of helenalin and related helenanolides with sulfhydryl containing biomolecules. Bioorg Med Chem 5:645–653

Singh B, Guru SK, Kour S, Jain SK, Sharma R, Sharma PR, Singh SK, Bhushan S, Bharate SB, Vishwakarma RA (2013) Synthesis, antiproliferative and apoptosis-inducing activity of thiazolo[5,4-*d*]pyrimidines. Eur J Med Chem 70:864–874

Taha M, Ismaila NH, Imrana S, Mohamada MH, Wadood A, Rahimd F, Saade SM, Rehman AU, Khane KM (2016) Synthesis, α-glucosidase inhibitory, cytotoxicity and docking studies of 2-aryl-7-methylbenzimidazoles. Bioorg Chem 65:100–109

Taha M, Shah SAA, Afifi M, Zulkeflee M, Sultan S, Wadood A, Rahim F, Ismail NH (2017) Morpholine hydrazone scaffold: synthesis, anticancer activity and docking studies. Chin Chem Lett 28:607–611

Vasas A, Hohmann J (2011) Xanthane sesquiterpenoids: structure, synthesis and biological activity. Nat Prod Rep 28:824–842

Winters TE, Geissman TA, Safir D (1969) Sesquiterpene lactones of *Xanthium species* Xanthanol and isoxanthanol, and correlation of xanthinin with ivalbin. J Org Chem 34:153–155

Woods JR, Mo H, Bieberich AA, Alavanja T, Colby DA (2013) Amino-derivatives of the sesquiterpene lactone class of natural products as prodrug. Med Chem Commun 4:27–33

Yoon JH, Lim HJ, Lee HJ, Kim HD, Jeon R, Ryu J-H (2008) Discovery of betrixaban (PRT054021), N-(5-chloropyridin-2-yl)-2-(4-(N,N-dimethylcarbamimidoyl)benzamido)-5-methoxybenzamide, a highly potent, selective, and orally efficacious factor Xa inhibitor. Bioorg Med Chem Lett 18:2179–2185

Zhang Q, Lu Y, Ding Y, Zhai J, Ji Q, Ma W, Yang M, Fan H, Long J, Tong Z, Shi Y, Jia Y, Han B, Zhang W, Qui C, Ma X, Li Q, Shi Q, Zhang H, Li D, Zhang J, Lin J, Li L, Gao Y, Chen Y (2012) Guaianolide sesquiterpene lactones, a source to discover agents that selectively inhibit acute myelogenous leukemia stem and progenitor cells. J Med Chem 55:8757–8769

Zhou J, Zhang Y (2008) Cancer stem cells: models, mechanisms and implications for improved treatment. Cell Cycle 7:1360–1370