Original Research Article

JOURNAL OF BASIC PHARMACOLOGY AND TOXICOLOGY

Synthesis and biological evaluation of sulphonyl derivatives of naturally occurring chromone alkaloid of rohitukine as anticancer agents

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Abstract

The present study deals with isolation of active constituent, rohitukine from the stem bark of *Dysoxylum binectariferum*, and synthesis of a series of its new sulphonyl derivatives aiming to enhance its therapeutic efficacy. Rohitukine has been isolated with more than 95% purity and ten new semisynthetic analogs have been prepared using different sulphonyl chlorides. These derivatives were evaluated for anticancer activity against MCF-7 (ER +ve) and MDA-MB-231 (ER -ve) breast cancer cell lines. Compounds K6, K8 and K10 showed significant activity against breast cancer cell lines at a concentration of 17.5 µM, 17 µM and 19 µM in MCF-7 and 20 µM, 9.8 µM and 28.5 µM in MDA-MB-231 respectively. These compounds induced significant apoptosis in MCF-cell line. Further work may enhance the anticancer activity.

Key words: Rohitukine, semisynthesis, Anticancer activity, *Dysoxylum binectariferum*, MCF-7, MDA-MB-231

1. Introduction

Cancer is a multi-factorial disease with excessive and robust biological networks and is a major public health burden of present era. In addition to developed countries, developing countries are also being increasingly afflicted with cancer, due to increased life expectancy and altered pattern of socio-cultural life dominated by western medicines that may lead to increased cancer risk [1]. It is thought to be caused by the interaction between genetic susceptibility and environmental factors. The characteristic feature of cancer cells include genetic instability, deregulation of multiple cellular signaling pathways, uncontrolled replication, avoidance of apoptosis, persistent angiogenesis, invasion of neighboring tissues and metastasis. Furthermore intra-tumoral heterogeneity caused due to genetic instability, results in adaptive resistance, therefore the survival rate of cancer patients remains very limited in many cases [2]. Cancer can be treated to some extent using chemotherapeutic agents with specific targets for example, the cell cycle. This has led to a search for materials that have specific targets in cancerous cells that control the cell cycle. In fact, compounds that target multiple intracellular components and distinct molecular mechanisms may be preferable and considered more promising.

Most of the anticancer drugs act through inducing apoptosis during mitotic arrest, otherwise after mitotic slippage following intrinsic pathway new that need stimulation of Mitochondrial Outer Membrane Permeabilization (MOMP) [3, 4]. In this regard, there is considerable interest in the design and development of molecules with higher efficacy, selective anticancer activity and human body tolerability. Accumulating evidence has shown that natural products and their semi-synthetic derivatives remain one of the major sources of potential anti-cancer agents with nearly 50% of the new chemical entities launched in the market [5, 6]. These are known to exert site-specific action on multiple cellular signaling pathways without causing undesired toxicity in normal cells. These non-toxic natural agents could be useful in combination with conventional chemotherapeutic agents for the treatment of human malignancies with lower toxicity and higher effectiveness. Nevertheless, proper characterization of naturally derived compounds particularly in view of cancer specific action without cytotoxic effects on normal cells is essential. Breast cancer is the most prevalent form of cancer and the second leading cause of deaths in women caused by cancer all over the world [7, 8]. Tamoxifen, an estrogen receptor (ER) agonist acting through ER receptor is the most effective anti-breast cancer molecule that acts by inhibiting estrogen action in ER +ve breast cancer [9, 10]. However, it is not effective against ER negative tumors. Therefore, there is an urgent need to develop novel agents with activity against estrogen-dependent as well as estrogen-independent breast cancer.

As part of our drug development programme from natural resources, we aimed to explore and to develop new anti-breast cancer molecules from indigenous medicinal plant *Dysoxylum binectariferum* Hook.f (Meliaceae). The stem bark of *D. binectariferum* is used as folk medicine for treatment of several diseases. Rohitukine, a benzopyranone motif containing chromone alkaloid is the major active constituent of this plant, showing significant biological activities [11, 12]. Benzopyranones are key structural motifs found in a large number of biologically active molecules from natural products [13, 14] that form a family of active compounds with a wide range of biological activities including antidiabetic, anti-inflammatory, anti-oesteogenic, antimicrobial, anti-allergic, antioxidant, antitumor and cytotoxic activities [15, 16]. Rohitukine has received significant attention for its medicinal activities leading to the synthesis of its semi-synthetic analogs. In this present study, we have investigated anticancer activity of new sulphonyl derivatives of rohitukine against MCF-7 (ER +ve) and MDA-MB-231 (ER -ve) breast cancer cell lines.

2. Materials and methods

2.1. Plant material

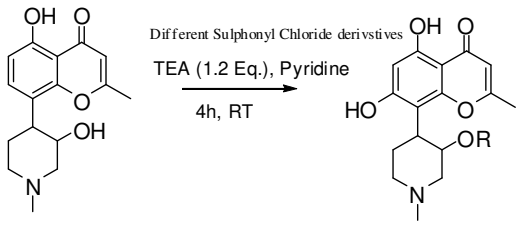
Stem bark of *D. binectariferum*, was collected from Sindhuburg, Maharashtra, India, and identified by the Botany Division of Central Drug Research Institute, Lucknow, India. A voucher specimen number 4032 has been kept in the Herbarium of the Institute.

2.2. Extraction/Fractionation/Isolation Procedure

Powdered air-dried ground stem bark (2.0 kg) was extracted with 95% v/v aqueous ethanol repeatedly (4 × 5 L) and the combined extracts were filtered and concentrated under reduced pressure below 50 °C to minimum volume of 1 L. It was further dried in hot air vacuum oven at 45 °C to brown powder (crude extract yield 44.2 g). The brown powder (32 g) was fractionated in to chloroform soluble (10.2 g) and chloroform insoluble fractions by maceration with chloroform. The chloroform fraction on repeated column chromatography over silica gel and final purification by HPLC on reverse phase C18 R.P columns using acetonitrile- water 55:45, v/v, flowrate-1.0 ml/min using UV detector (230 nm) yielded Rohitukine (1.2%) as major constituent, which was characterized by using NMR, mass, derivatization and comparing the data with those reported in literature [12].

2.3. General procedure for the synthesis of compound K1-K10

Rohitukine was stirred in the pyridine triethylamine (6:2) solution at 40 °C for 1 hr. Substituted sulfonyl chlorides were added to this stirring solution and reaction was continued for 4 hr. The reaction mixture was neutralized with 1 N HCl and extracted with dichloromethane. The reaction mixture was washed with water (20 mL x 2), brine solution (20 mL x 2) and filtered over dried anhydrous Na_2SO_4. The reaction mixtures were evaporated *in vacuo* and residue were purified with column chromatography on SiO_2 (100-200 mesh), affording compound K1 to K10 (Scheme 1). These sulfonyl derivatives were synthesized in moderate to good yield (Table 1).



Scheme 1

K-1 [4-(5,7-dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl)-1-methylpiperidin-3-yl 2-nitro benzenesulfonate]: Compound K-1 was prepared from rohitukine and 2-nitrobenzene-1-sulfonylchloride using representative procedure to yield pure compound through elution with 80% chloroform in hexane. Yield: 81%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ: 12.95 (s, 1H), 8.0-7.50 (m, 4H), 6.50 (s, 1H), 6.28 (s, 1H), 4.96-4.90 (m, 1H), 3.06-2.99 (m, 1H), 2.89-2.81 (m, 3H), 2.55-251 (m, 1H), 2.31 (s, 3H), 2.25 (s, 3H), 2.00-192 (m, 1H), 1.47-1.43 (m, 1H); ^{13}C NMR (200 MHz, $CDCl_3$) δ: 183.81, 166.26, 162.26, 161.86, 161.04, 151.14, 138.38, 137.15, 132.16, 130.60, 128.12, 111.32, 109.82, 106.76, 100.04, 68.02, 56.94, 55.16, 42.17, 27.10, 25.11, 20.78; ESI-MS: m/z 490 $(M+1)^+$

K-2 [4-(5,7-dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl) -1-methylpiperidin-3-yl 4-nitrobenzenesulfonate]: Compound K-2 was prepared from rohitukine and 4-nitrobenzene-1-sulfonylchloride using representative procedure to afford pure compound through elution with 80% chloroform in hexane. Yield: 72%; 1H NMR: (300 MHz, CDCl3) δ: 13.16 (s, 1H), 8.19 (d, J = 9.2 Hz, 2H), 7.87 (d, J = 9.2 Hz, 2H), 6.59 (s, 1H), 6.17 (s, 1H), 5.02-4.95 (m, 1H), 3.18-3.11 (m, 1H), 2.80-2.73 (m, 3H), 2.62-2.59 (m, 1H), 2.54 (s, 3H), 2.38 (s, 3H), 1.84-1.79 (m, 1H), 1.76-1.72 (m, 1H); 13C NMR (200 MHz, CDCl3) δ: 182.91, 166.29, 160.49, 160.16, 159.41, 155.14, 152.95, 132.15, 131.98, 125.14, 125.08, 111.25, 110.40, 106.04, 97.24, 67.11, 57.92, 57.22, 43.12, 26.34, 24.82, 20.22; ESI-MS: m/z 490 (M+1)+

K-3 [4-(5,7-dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl) -1-methylpiperidin-3-yl 3-nitrobenzenesulfonate]: Compound K-3 was prepared from rohitukine and 4-nitrobenzene-1-sulfonylchloride using representative procedure to afford pure compound through elution with 90% chloroform in hexane. Yield: 76%; 1H NMR: (300 MHz, CDCl3) δ: 12.90 (s, 1H), 8.48 (s, 1H), 8.12 (d, J = 9 Hz, 1H), 7. 87 (d, J = 9 Hz, 1H), 7.62-7.58 (m,1H), 6.30 (s, 1H), 6.00 (s, 1H), 5.10-5.04 (m, 1H), 3.10-3.03 (m, 1H), 2.72-2.66 (m, 3H), 2.59-2.55 (m, 1H), 2.48 (s, 3H), 2.34 (s, 3H), 1.72-1.68 (m, 1H), 1.60-1.54 (m, 1H); 13C NMR (200 MHz, CDCl3) δ: 184.51, 168.59, 163.24, 162.56,161.84, 152.14, 144.26, 138.82, 132.24, 132.12, 128.24, 112.08,

110.40, 107.11, 99.24, 67.38, 57.44, 56.82, 42.12, 26.84, 26.32, 22.38; ESI-MS: m/z 490 (M+1)+

K-4 [4-(5,7-dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl) -1-methylpiperidin-3-yl 4-chlorobenzenesulfonate]: Compound K-4 was prepared from rohitukine and 4-chlorobenzene-1-sulfonylchloride using representative procedure to afford pure compound through elution with 80% chloroform in hexane. Yield: 68%; 1H NMR: (300 MHz, CDCl3) 12.85 (s, 1H), 7.58 (d, J = 7.8 Hz, 2H), 7.47 (d, J = 7.8 Hz, 2H), 6.44 (s, 1H), 6.25 (s, 1H), 4.89-4.82 (m, 1H), 2.94-2.87 (m, 1H), 2.68-2-64 (m, 3H), 2.62-2.58 (m, 1H), 2.30 (s, 3H), 2.26 (s, 3H), 2.00-192 (m, 1H), 1.78-1.74 (m, 1H); 13C NMR: (200 MHz, CDCl3) δ 183.64, 165.96, 162.10, 161.65, 161.02, 150.24, 139.18, 133.25, 132.95, 130.60, 130.24, 110.94, 109.67, 106.24, 99.74, 67.34, 57.98, 56.38, 43.10, 26.10, 25.85, 20.78; ESI-MS: m/z 479 (M+1)+

K-5 [4-(5,7-dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl) -1-methylpiperidin-3-yl 2,4-dichlorobenzenesulfonate]: Compound K-5 was prepared from rohitukine and 2,4-dichlorobenzene-1-sulfonylchloride using representative procedure to afford pure compound at 90% chloroform in hexane as eluent. Yield: 75%; 1H NMR (300 MHz, CDCl3): δ: 13.02 (s, 1H), 8.04 (s, 1H), 7.85 (d, J = 9 Hz, 1H), 7.78 (d, J = 9 Hz, 1H), 6.28 (s, 1H), 6.14 (s, 1H), 5.02-4.97 (m, 1H), 3.18-3.11 (m, 1H) 2.68-2-59 (m, 3H), 2.57-2.54 (m, 1H), 2.41 (s, 3H), 2.34 (s, 3H), 1.87-1.70 (m, 2H), 13C NMR (200 MHz, CDCl3) δ: 183.42, 167.29, 162.4, 161.6, 158.41, 140.62, 138.23, 135.4, 134.34, 131.19, 129.24,112.78, 110.65, 107.26, 98.84, 68.57, 57.92, 56.24, 43.47, 27.3, 25.12, 19.98; ESI-MS: m/z 513 (M+1)+

K-6 [4-(5,7-dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl)-1-methylpiperidin-3-yl 4-methyl benzenesulfonate]: Compound K-6 was prepared from rohitukine and 4-methylbenzene-1-sulfonylchloride using representative procedure to afford pure compound at 80% chloroform in hexane as eluent. Yield: 70%; 1H NMR (300 MHz, CDCl3) δ: 12.90 (s, 1H), 7.73 (d, J = 6 Hz, 2H), 7.44 (d, J = 6 Hz, 2H), 6.33 (s, 1H), 6.02 (s, 1H), 4.98-4.92 (m, 1H), 3.10-3.06 (m, 1H), 2.96-2.89 (m, 3H), 2.41 (s, 3H), 2.35 (s, 3H), 2.30 (s, 3H), 2.25-2.21 (m, 1H), 1.84-171 (m, 2H); 13C NMR (200 MHz, CDCl3) δ: 182.94, 166.40, 162.92, 161.46, 158.62, 145.24, 142.25, 131.15, 130.98, 130.24, 130.04, 112.02, 110.90, 107.08, 99.04, 68.10, 58.96, 57.92, 43.12, 25.23, 24.84 22.56, 20.12; ESI-MS: m/z 459 (M+1)+

K-7 [4-(5,7-dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl)-1-methylpiperidin-3-yl 2,4,6-trimethylbenzenesulfonate]: Compound K-7 was prepared from rohitukine and 2,4,6-trimethylbenzene-1-sulfonyl chloride using representative procedure to afford pure compound at 90% chloroform in hexane as eluent. Yield: 68%; 1H NMR (300 MHz, CDCl3) δ: 13.06, 7.10 (s, 2H), 6.29 (s, 1H), 6.02 (s, 1H), 4.96-4.91 (m, 1H), 3.16-3.10 (m, 1H), 2.81-2.76 (m, 3H), 2.56 (s, 6H), 2.46-2.42 (m, 1H), 2.40 (s, 3H), 2.35 (s, 3H), 2.29 (s, 3H), 1.90-1.82 (m, 2H), 13C NMR (200 MHz, CDCl3) δ:184.12, 166.72, 163.24, 163.12, 161.24, 142.12, 138.84, 137.23, 137.19, 130.42, 130.40, 112.78, 110.65, 105.82, 96.48, 67.95, 63.22, 5812, 56.82, 44.38, 24.42, 23.24, 23.21, 22.12, 19.44; ESI-MS: m/z 487 (M+1)+

K-8 [4-(5,7-dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl)-1-methylpiperidin-3-yl 2,4,6-triisopropylbenzenesulfonate]: Compound K-8 was prepared from rohitukine and 2,4,6-triisopropylbenzene-1-sulfonyl chloride using representative procedure to afford pure compound at 80% chloroform in hexane as eluent. Yield: 77%; 1H NMR (300 MHz, CDCl3) δ: 12.98 (s, 1H), 7.60 (s, 2H), 6.20 (s, 1H), 6.04 (s, 1H), 5.02-4.96 (m, 1H), 3.21-3.14 (m, 2H) 2.82-2.64 (m, 6H), 2.36 (s, 3H), 2.29 (s, 3H), 1.84-1.75 (m, 2H) 1.30 (s, 6H), 1.27 (s, 6H), 1.24 (s, 6H),13C NMR (200 MHz, CDCl3) δ:184.12, 167.82, 162.44, 162.16, 160.10, 156.12, 156.08, 154.15, 126.24, 124.82, 124.77, 111.78, 110.65, 106.12, 99.48, 67.15, 59.42, 57.22, 43.12, 36.46, 36.44, 35.82, 26.12, 25.90, 24.80, 24.77, 24.42, 24.40, 24.04, 24.02, 21.24; ESI-MS: m/z 571 (M+1)+

K-9 [4-(5,7-dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl) -1-methylpiperidin-3-yl 4-methoxy benzenesulfonate]: Compound K-9 was prepared from rohitukine and 4-methoxybenzene-1-sulfonyl chloride using representative procedure to afford pure compound at 80% chloroform in hexane as eluent. Yield: 74%; 1H NMR (300 MHz, CDCl3) δ: 12.98 (s, 1H), 7.42 (d, J = 8.52 Hz, 2H), 6.96 (d, J = 8.52 Hz, 2H), 6.23 (s, 1H), 6.05 (s, 1H), 4.97-4.91 (m, 1H), 3.37 (s, 3H), 3.18-3.10 (m, 1H), 2.74-2.66 (m, 3H), 2.41 (s, 3H), 2.35 (s, 3H), 2.29-2.25 (m, 1H), 2.02-184 (m, 2H); 13C NMR (200 MHz, CDCl3) δ: 185.22, 168.48, 165.94, 160.59, 160.16,159.12, 141.67, 135.12, 135.12, 117.28, 117.28, 112.04, 110.02, 106.12, 99.14, 67.17, 58.74, 56.34, 55.40, 43.87, 25.74, 25.24 21.28; ESI-MS: m/z 475 (M+1)+

K-10 [4-(5,7-dihydroxy-2-methyl-4-oxo-4H-chromen-8-yl) -1-methylpiperidin-3-yl naphthalene-1-sulfonate]: Compound K-10 was prepared from rohitukine and 4 naphthalene-1-sulfonyl chloride through representative procedure to afford pure compound at 90% chloroform in hexane as eluent. Yield: 80%; 1H NMR (300 MHz, CDCl3) δ: 12.90 (s, 1H), 8.70 (d, J=8.49, 1H), 8.13 (d, J=7.71, 1H), 8. 11 (d, J=6.96, 1H), 7.94 (d, J=8.01, 1H) 7.71, (t, J=7.92), 7.63-7.49 (m, 2H) 6.10 (s), 5.98 (s), 4.95-4.90 (m, 1H,), 3.07-3.01 (m, 1H), 2.74-2.68 (m, 3H), 2.57-2.52 (m, 1H), 2.32 (s, 3H), 2.26 (s, 3H) 2.12-1.96 (m, 2H); 13C NMR (200 MHz, CDCl3) δ:183.22, 167.35, 163.23, 163.02, 160.46, 144.74, 135.91, 132.82, 130.80, 130.14, 128.82, 128.71, 126.90, 126.74, 125.51, 111.72, 110.10, 106.86, 98.94,69.06, 58.22, 55.12, 42.32, 25.83, 25.24, 20.54; ESI-MS: m/z 495 (M+1)+

2.4. Cell viability assay

MCF-7 and MDA-MB-231 cell lines were used for screening of anti-breast cancer activity. Cell viability was evaluated by the trypan blue exclusion method. Cells treated with or without the test compounds were harvested by trypsinization. Cells were incubated in 0.04% trypan blue (Sigma-Aldrich) for 4 min and counted under a hemocytometer. The total number of cells and number of cells that retained the dye (nonviable cells) were calculated.

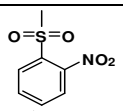
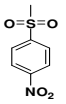
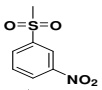
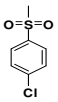
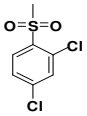
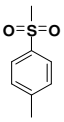
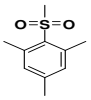
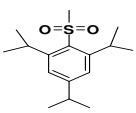
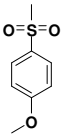
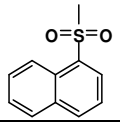
2.5. Antiproliferative activity assay

The antiproliferative effect was determined through MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay by the method published by Botta [17]. 1 × 10^4 cells/well were seeded in 96-well microculture plates in 100 µL DMEM, media supplemented with 10% FBS in each well and incubated in a CO_2 incubator for 24 hr at 37 °C. All the compounds were dissolved in DMSO to prepare 10 mM stock solutions and subsequently diluted to the desired concentrations in the culture medium in the wells with respect to the vehicle control. After 48 hr of incubation, media were removed and 100 µL MTT (5 mg/mL) was added to each well and plates were further incubated for 4 hr. Supernatant was removed carefully from each well, 100 µL of DMSO was added to each well to dissolve formazon crystals and the absorbance was recorded at 570 nm using microtiter reader (Bio-Tek, USA).

2.6. Analysis of mitochondrial membrane potential (MMP)

The MMP was measured by the uptake of unique fluorescent cationic dye, JC-1 (excitation at 488 nm and emission at 525 nm), to signal the loss of MMP [17]. This fluorescent probe exists as a green fluorescent monomer (emission 527 nm) at low MMP. Mitochondrial depolarization is indicated by an increase in green fluorescence (FL-1). The MCF-7 cells (0.2 × 10^6 cells) were seeded in a 6-well plate and exposed to compounds at varying concentrations for 36 hr. After that the cells were washed and finally harvested in chilled PBS containing JC-1 (1 µM). The samples were incubated at 37 °C for 30 min in the dark, washed twice with chilled PBS and finally resuspended in 200 µL PBS. Mitochondrial permeability transition was subsequently quantified on FACS.

Table 1. Percentage yield of different sulphonyl derivatives of rohitukine

Compound	R	Molecular formula	Molecular weight	Yield%
K-1		$C_{22}H_{22}N_2O_9S$	490	81%
K-2		$C_{22}H_{22}N_2O_9S$	490	72%
K-3		$C_{22}H_{22}N_2O_9S$	490	76%
K-4		$C_{22}H_{22}ClNO_7S$	479	68%
K-5		$C_{22}H_{21}Cl_2NO_7S$	513	75%
K-6		$C_{23}H_{25}NO_7S$	459	70%
K-7		$C_{25}H_{29}NO_7S$	487	68%
K-8		$C_{31}H_{41}NO_7S$	571	77%
K-9		$C_{23}H_{25}NO_8S$	475	74%
K-10		$C_{26}H_{25}NO_7S$	495	80%

2.7. Assessment of apoptosis by Flow Cytometry

The percentage of cells undergoing apoptosis was determined using Annexin V FITC Assay kit (BD Biosciences). Flurochrome-labeled Annexin V is used for the detection of exposed phosphatidylserine (PS) and propidium iodide (PI) for the differentiation of necrotic cells using flow cytometry. The MCF-7 cells were cultured and treated with different doses of compounds for 24 hr. At the end of this time, period cells were washed twice with PBS, collected in 1X binding buffer and centrifuged at 1200 rpm for 5 min. Afterwards, the cell pellet was treated with 5 µL Annexin V and 5 µL of PI, incubated for 15 min. in dark. Finally, 200 µL of binding buffer was added to the cell pellet and analyzed by flow cytometry (BD Biosciences) [18].

2.8. Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) with Newman–Keüls posthoc test using

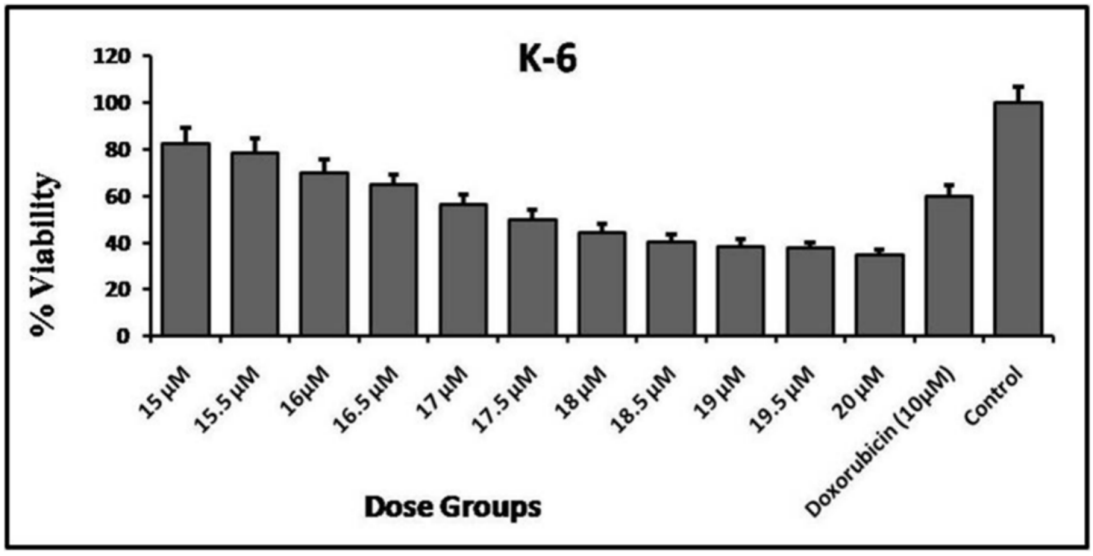


Figure 1(a). Assessment of IC50 value of compound K-6 in MCF-7 cells

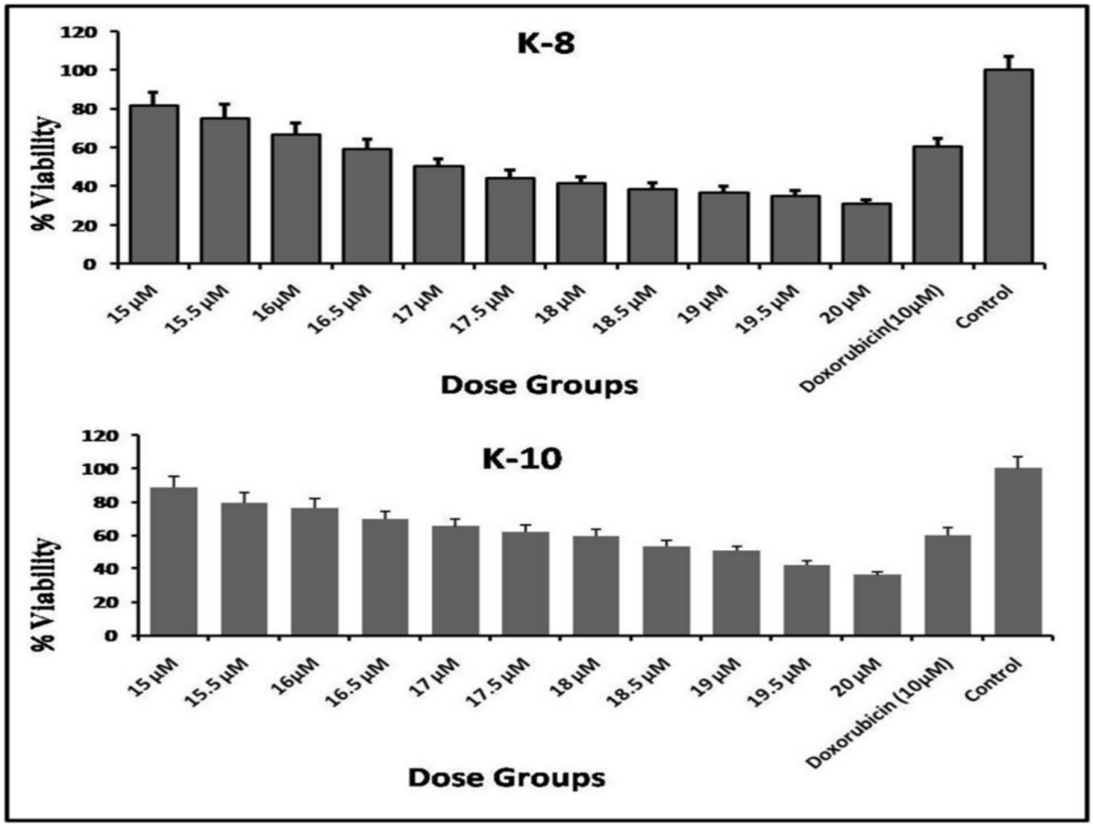


Figure 1 (b). Assessment of IC50 values of compound K-8 and K-10 in MCF-7 cell

GraphPad Prism version 5.00 and $p < 0.05$ was considered statistically significant.

3. Results and discussion

We embark to synthesize new semisynthetic derivatives of the lead molecule rohitukine in order to explore the anticancer effect of different substituted sulphonyl chlorides. Compounds K1-K10 were synthesized in moderate to good yield (Table 1) and evaluated for antiproliferative activity against MCF-7 and MDA-MB-231 cell lines alongside doxorubicin as standard drug.

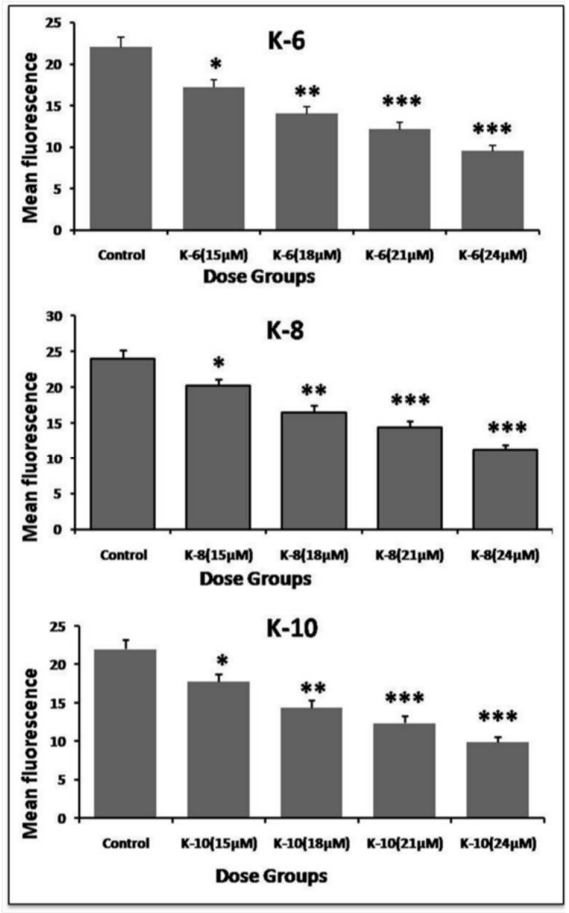


Figure 2. Effect of compounds K-6, K-8 and K-10 on loss of MMP. Trypsinized cells were incubated with the fluorescent cationic dye, JC-1 (excitation at 488 nm and emission at 525 nm) (1 μM) for 30 min at 37 °C in the dark, washed twice with chilled PBS and finally resuspended in 200 μL PBS. The mitochondrial permeability transition was quantified on FACS. Data shown are the mean ± SE of one of three similar experiments each performed in triplicate *p < 0.05, **p < 0.01, ***p < 0.001 when compared with control group.

Compounds K-6, K-8 and K-10 showed promising antiproliferative activity showing minimum IC_{50} values against MCF-7. IC_{50} of K-6, K-8 and K-10 are 17.5, 17.0 and 19.0 μM respectively, these compounds also showed antiproliferative potential with IC_{50} values 20 μM, 19.8 μM and 28.5 μM respectively against MDA-MB-231. The cytotoxic activities for compounds K-6, K-8 and K-10 against MCF-7 have been presented in figure 1(a) and 1(b).

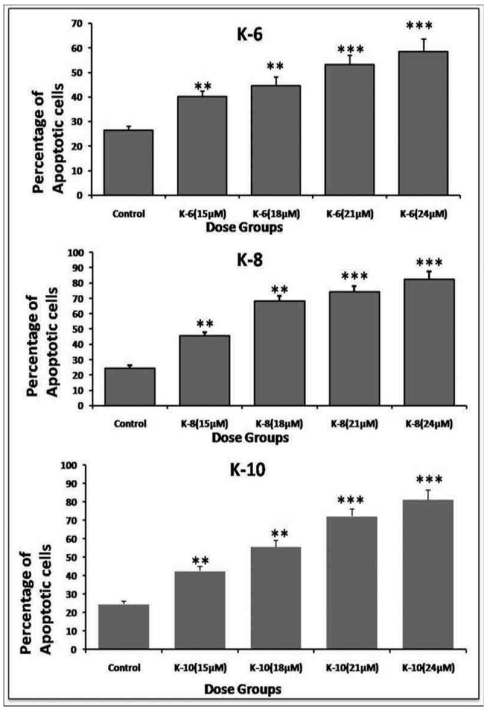


Figure 3. Assessment of apoptosis - percentage of apoptotic cells of MCF-7 cells followed by treatment with compounds K-6, K-8 and K-10 at 15μM, 18μM, 21 μM and 24 μM concentrations. Control indicates the untreated cells. Data shown are the mean ± SE of one of three similar experiments each performed in triplicate; **p < 0.01, ***p < 0.001 when compared with control group.

Thus, these semisynthetic analogs of rohitukine have been found to be effective against both breast cancer cell lines and this is the rationale to further evaluate various parameters to determine the mechanism of action of these derivatives. The compounds K-6, K-8 and K-10 showed significant decrease in mitochondrial membrane potential in a dose-dependent manner which is reflected by decrease in mean fluorescence with respect to control (Figure 2). Percentage of cells undergoing apoptosis was determined using flow cytometry. Treatment of MCF-7 cells with compounds K-6, K-8 and K-10 showed induction of apoptosis in a dose-dependent manner (Figure 3). *Dysoxylum binectariferum* stem bark as well as its major active constituent rohitukine possess diverse biological activities. However, for the first time its sulphonyl derivatives, compounds K-6, K-8 and K-10, have been evaluated for anticancer activity and have shown significant results. The mechanism of anticancer activity was by induction of apoptosis.

4. Conclusion

The synthesis of these derivatives from rohitukine have served their purpose to an extent and taking lead from them, other derivatives with better potential as well as novel mechanism of action can be derived to combat cancer.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

Authors are thankful to the Director, CDRI, Lucknow, India for constant encouragement for the drug developing program on anticancer agents and Council of Scientific and Industrial Research for senior research fellowship. We are grateful to the sophisticated analytical instrument facility (CDRI) for spectral data.

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