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Synthesis and Anticancer Activity of Some Novel 1,3-Diaryl/heteroarylprop-2-en-1-one Derivatives

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In the present investigation, a series of some novel 1,3-diaryl/heteroarylprop-2-en-1-one derivatives (**3a-j**) have been synthesized and evaluated for their *in vitro* cytotoxicity against three cancer cell lines, two hepatocarcinoma cell lines HUH-7, Hep-3b and one leukemia cancer cell line MOLT-4. Based on these results, structure-activity relationship (SAR) was studied on modification of R^1 and R^2 to identify the compound with maximum potency. Amongst the compounds, **3b** and **3d** strongly inhibited the growth of Hep-3b and MOLT-4 cells with IC_{50} value of 3.39 and 3.63 μM respectively. The results obtained from the inhibitory study had further been supported by the reactive oxygen species (ROS) measurement using flow cytometry in MOLT-4 cells. These observations collectively reveal that compounds comprising 1,3-diarylprop-2-en-1-one framework with pyrazole ring at position-3 and heteroaryl/aryl substituents at position-1 can be used as promising anti-cancer agents.

Key Words : 1,3-Diaryl/heteroarylprop-2-en-1-one, Pyrazole, Anti-cancer, Reactive oxygen species

Introduction

Cancer is one of the major causes of death worldwide.[1] Among different kinds of cancers, hepatic cancer and leukemia cannot be cured easily due to the lesser number of therapeutics available in the market. Therefore, there is an impetus in the field of medicinal chemistry to synthesize and develop more potent therapeutic agents with less toxicity, and safer therapeutic profile for such type of cancers. Many clinically successful anticancer drugs are either natural products or have been developed from naturally-occurring lead compounds such as taxol, *etc*.[2-6] In literature, it has been reported that synthetic derivatives are often found to be more active than parent compounds.[7]

Azole and propeneone derivatives have drawn attention very much for their biological and chemotherapeutic importance. Compounds having pyrazole moiety are known to exhibit different types of anti-cancer activities besides other biological properties like CNS depressant, neuroleptic, tuberculostatic, antihypertensive, antileishmanial, analgesic, anti-diabetic, anti-tumor and anti-microbial activities.[8-10] Moreover, the natural or synthetic compounds bearing prop-2-en-1-one framework is another interesting class since they constitute an important group of active agents and serve as precursors for the synthesis of various classes of heterocycles.[11] They have been extensively reported to exhibit a wide variety of biological properties such as anti-cancer,[12] anti-inflammatory,[13] anti-microbial,[14] anti-hepatotoxic,[15] anti-oxidant,[16] anti-malarial,[17] and anti-tuberculosis,[18] activities. Recently, we reported the synthesis of a series of some novel prop-2-en-1-ones, which were found to have anti-influenza viral activities.[19] So far, the major concern of the researchers has been confined to anticancer activities of prop-2-en-1-ones having aryl or heteroaryl moiety at position-3 with differently substituted aryl groups at position-1 but reverse of the substitution pattern on the same propenone system has not been explored well before. Thus, prop-2-en-1-one derivatives having a pyrazole moiety at position-3 along with various heteroaryl or aryl groups at position-1 could be of significant interest and potential target compounds.[19] Encouraged by the previous findings reported in literature for both pyrazole and prop-2-en-1-one systems individually with structural diversity and in continuation of our ongoing interest in developing more effective anti-cancer therapeutics, we designed and synthesized a series of some novel prop-2-en-1-one derivatives bearing heteroaryl/aryl moieties at position-1 with pyrazole ring at position-3. All the synthesized compounds were evaluated for their anti-cancer activities against three cancer cell lines, two hepatocarcinoma cell lines (HuH-7, Hep-3b) and one leukemia cancer cell line (MOLT-4). The extent of apoptosis of the compounds was further measured by reactive oxygen species (ROS) studies in MOLT-4 cell using flow cytometry.

Experimental

Chemistry. Melting points were determined in open capillary tubes and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on JNM-ECA 500 spectrophotometer (Jeol) at 500 and 125 MHz, respectively. The internal standard

used for these spectra was TMS. IR spectra (KBr) were recorded on a Bruker-Vector 22 instrument. Molecular mass of the compounds were derived from HRMS technique using JEOL JMS600 instrument. The stationary phases used for column chromatography (Silica gel 60, 70-230 mesh), and TLC plates (Silica-gel 60 F_{254}) were purchased form Merck KGaA. Spots were visualized under UV radiations. All the reagent-grade chemicals were purchased from the Sigma-Aldrich (St. Louis, MO, USA).

Synthesis of 1-(Thien-2-yl)-3-(3-methyl-1-phenyl-1*H*-pyrazole-4-yl)prop-2-en-1-one (3a). To a mixture of 2-acetylthiophene (1 mL, 0.0092 mol) and 3-methyl-1-phenyl-1*H*-pyrazole-4-yl (1.72 g, 0.0092 mol) in ethanol was added aqueous sodium hydroxide (0.44 g, 0.011 mol) at 0 ºC. The resulting reaction mixture was allowed to stir for 6-8 h at room temperature. The reaction was monitored by TLC and on completion, reaction mixture was poured into ice-cold water. Then pH of the mixture adjusted to 6 using 0.01 N HCl solution. The obtained precipitates were filtered, dried and recrystallized from ethanol to get the compound **3a**. Remaining compounds (**3b-j**) were prepared according to this procedure.

1-(Thien-2-yl)-3-(3-methyl-1-phenyl-1*H*-pyrazole-4-yl)-prop-2-en-1-one (3a). Yield 92%; IR (ν_{max}, KBr): 1636 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.52 (3H, s), 7.19 (2H, m), 7.31 (1H, m), 7.46 (2H, m), 7.68 (3H, m), 7.82 (2H, m), 8.19 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.2, 118.5, 119.1, 119.9, 127.0, 127.4, 128.3, 129.6, 131.4, 133.6, 134.5, 139.4, 145.7, 151.0, 182.0; HRMS (FAB+): *m/z* 294.0911 (M^+), $C_{17}H_{14}N_2OS$ requires 294.0827.

1-Phenyl-3-(3-methyl-1-phenyl-1*H*-pyrazole-4-yl)prop-2en-1-one (3b). Yield 83%; IR (ν_{max}, KBr): 1641 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.52 (3H, s), 7.31 (2H, m), 7.47 (3H, m), 7.56 (2H, m), 7.68 (2H, m), 7.80 (1H, d, *J* = 15.7 Hz), 8.01 (2H, m), 8.18 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.3, 118.7, 119.1, 120.2, 127.0, 127.4, 128.4, 128.7, 129.6, 132.7, 135.3, 138.4, 139.4, 151.0, 190.3; HRMS (FAB+): *m/z* 288.1349 (M^+), $C_{19}H_{16}N_2O$ requires 288.1263.

1-(4-Hydroxy-6-methyl-2*H*-pyran-3-yl)-3-(3-methyl-1-phenyl-1*H*-pyrazole-4-yl)prop-2-en-1-one (3c). Yield 40%; IR (ν_{max}, KBr): 1636 cm^{-1} (C=O), 1735 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.26 (3H, s), 2.52 (3H, s), 5.94 (1H, m), 7.31 (1H, m), 7.45 (2H, m), 7.69 (2H, m), 7.95 (1H, d, *J* = 15.7 Hz), 8.08 (1H, d, *J* = 15.7Hz), 8.25 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.0, 20.7, 98.8, 102.7, 119.1, 119.2, 120.7, 127.1, 128.1, 129.6, 137.0, 139.3, 151.7, 161.5, 168.3, 183.4, 192.2; HRMS (FAB+): *m/z* 336.1188 (M^+), $C_{19}H_{16}N_2O_4$ requires 336.1110.

1-(5-Methylfuran-2-yl)-3-(3-methyl-1-phenyl-1*H*-pyrazole-4-yl)prop-2-en-1-one (3d). Yield 50%; IR (ν_{max}, KBr): 1644 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.44 (3H, s), 2.52 (3H, s), 6.21 (1H, m), 7.16 (1H, d, *J* = 16.0 Hz), 7.22 (1H, d, *J* = 3.4 Hz), 7.31 (1H, m), 7.46 (2H, m), 7.68 (2H, m), 7.81 (1H, d, *J* = 15.7 Hz), 8.18 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.1, 14.2, 109.3, 118.7, 119.1, 119.6, 126.9, 127.2, 129.3, 129.5, 133.6, 139.4, 151.0, 152.6, 157.9, 177.3; HRMS (FAB+): *m/z* 292.1212 (M^+), $C_{18}H_{16}N_2O_2$ requires 292.1290.

1-(2*H*-Chromen-2-one-3-yl)-3-(3-methyl-1-phenyl-1*H*-pyrazole-4-yl)prop-2-en-1-one (3e). Yield 87%; IR (ν_{max}, KBr): 1636 cm^{-1} (C=O), 1729 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.51 (3H, s), 7.34 (3H, m), 7.45 (2H, m), 7.66 (4H, m), 7.75 (1H, d, *J* = 15.7 Hz), 7.85 (1H, d, *J* = 15.7 Hz), 8.22 (1H, s), 8.59 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.0, 116.6, 118.5, 118.8, 119.0, 122.0, 124.9, 125.2, 126.9, 127.6, 129.4, 129.9, 134.1, 135.4, 139.2, 148.0, 151.4, 155.1, 159.4, 185.8; HRMS (FAB+): *m/z* 356.1234 (M^+), $C_{22}H_{16}N_2O_3$ requires 356.1161.

1-(4-Fluorophenyl)-3-(3-methyl-1-phenyl-1*H*-pyrazole-4-yl)prop-2-en-1-one (3f). Yield 67%; IR (ν_{max}, KBr): 1634 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.52 (3H, s), 7.16 (2H, m), 7.31 (2H. m), 7.46 (2H, m), 7.67 (2H, m), 7.80 (1H, d, *J* = 15.7 Hz), 8.03 (2H, m), 8.18 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.3, 115.7(d, $J^2_{C\text{-}F}$ = 23.2 five) 118.6, 119.1, 119.7, 127.0, 127.4 (d, $J^2_{C\text{-}F}$ = 8.2 five) 129.6, 130.9, 134.7, 135.5, 139.4, 151.0, 164.2 (d, $J^1_{C\text{-}F}$ = 24 six. 7 five) 188.6; HRMS (FAB+): *m/z* 306.1247 (M^+), $C_{19}H_{15}FN_2O$ requires 306.1168.

1-(5-Bromofuran-2-yl)-3-(3-methyl-1-phenyl-1*H*-pyrazole-4-yl)prop-2-en-1-one (3g). Yield 93%; IR (ν_{max}, KBr): 1644 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.51 (3H, s), 6.52 (1H, d, *J* = 3.4 Hz), 7.16 (1H, d, *J* = 15.7 Hz), 7.24 (1H, m), 7.30 (1H, m), 7.45 (2H, m), 7.68 (2H, m), 7.82 (1H, d, *J* = 15.7 Hz), 8.21 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.1, 114.7, 118.6, 118.9, 119.2, 127.0, 127.4, 127.7, 129.3, 129.6, 135.0, 139.4, 151.3, 155.6, 176.8; HRMS (FAB+): *m/z* 356.0239 (M^+) and 356.0160 ($M^+ + 2$) in the ratio showing typical bromine isotope profile (1:1), $C_{17}H_{13}BrN_2O_2$ requires 356.0160.

1-(4-Nitrophenyl)-3-(3-methyl-1-phenyl-1*H*-pyrazole-4-yl)prop-2-en-1-one (3h). Yield 67%; IR (ν_{max}, KBr): 1647 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.42 (3H, s), 7.32 (1H, m), 7.51 (2H, m), 7.62 (1H, d, *J* = 15.4 Hz), 7.70 (1H, d, *J* = 15.4 Hz), 7.79 (2H, d, *J* = 8.0 Hz), 8.25 (2H, d, *J* = 8.6 Hz), 8.36 (2H, d, *J* = 8.5 Hz), 9.1 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.8, 118.7, 118.9, 119.7, 124.1, 124.7, 127.0, 129.3, 129.9, 136.6, 143.2, 150.1, 151.0, 158.7, 187.5; HRMS (FAB+): *m/z* 333.1192 (M^+), $C_{19}H_{15}N_3O_3$ requires 333.1113.

1-(4-Fluorophenyl)-3-(1,3-diphenyl-1*H*-pyrazole-4-yl)-prop-2-en-1-one (3i). Yield 67%; IR (ν_{max}, KBr): 1634 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.14 (2H, m), 7.33 (2H, m), 7.48 (5H, m), 7.69 (2H, m), 7.79 (2H, m), 7.89 (1H, d, *J* = 15.4 Hz), 7.97 (2H, m), 8.35 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 115.6, 115.8 (d, $J^2_{C\text{-}F}$ = 23.2 five) 118.3, 119.4, 121.0, 126.9, 127.4 (d, $J^2_{C\text{-}F}$ = 8.2 five) 128.8, 129.6, 130.9, 131.0, 132.3, 134.6, 135.6, 139.4, 153.9, 166.5 (d, $J^1_{C\text{-}F}$ = 24 six. 7 five) 188.4; HRMS (FAB+): *m/z* 368.1403 (M^+), $C_{24}H_{17}FN_2O$ requires 368.1325.

1-(5-Methylfuran-2-yl)-3-(1,3-diphenyl-1*H*-pyrazole-4-yl)prop-2-en-1-one (3j). Yield 79%; IR (ν_{max}, KBr): 1642 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.41 (3H, s), 6.17 (1H, m), 7.12 (1H, d, *J* = 3.4 Hz), 7.19 (1H, d, *J* = 15.7

Hz), 7.33 (1H, m), 7.46 (5H, m), 7.69 (2H, m), 7.79 (2H, m), 7.89 (1H, d, J = 15.7 Hz), 8.33 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 109.3, 118.4, 119.1, 119.4, 121.1, 126.8, 127.2, 128.7, 128.8, 128.9, 129.6, 132.4, 133.8, 139.5, 152.6, 152.8, 152.9, 177.2; HRMS (FAB+): *m/z* 354.1447 (M^+),$C_{23}H_{18}N_2O_2$ requires 354.1368.

Anticancer Activity

MTT Assay.

Chemicals and Reagents: 3-(4,5-Dimethyl-2-thiazol-yl-2)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), Dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO) (HPLC grade) were purchased from Sigma (St. Louis, MO).

Cell Culture. In the present investigation, two hepatic cancer cell lines HUH-7, Hep-3b and leukemia cancer cell line MOLT-4 were used to study anti-cancer properties of compounds (**3a-j**). HUH-7 and Hep-3b were maintained in a monolayer at 37 °C in a humidified atmosphere of 5% CO_2 in DMEM with 10% FBS, penicillin (100 units/mL) and streptomycin (100 μg/mL). Leukemia cancer cell line (MOLT-4) was maintained at 37 °C in a humidified atmosphere of 5% CO_2 in RPMI-1640 with 10% FBS, penicillin (100 units/mL) and streptomycin (100 μg/mL).

Sample Preparation. Compounds **3a**, **3b**, **3d**, **3f**, **3g**, **3i** and **3j** were dissolved in DMSO while **3c**, **3e** and **3h** were dissolved in DMF to prepare the stock concentrations (100 μM). Stock solutions were further diluted with hepatic and leukemia growth medium. The final concentration of solvent in the growth medium was < 0.5%. All the steps were conducted in flow chambers.

MTT Cell Proliferation Assay. The inhibitory effect of compounds **3a-j** on growth of HUH-7, Hep-3b and MOLT-4 cells were measured by employing MTT colorimetric method.[21] Briefly, cells were plated in 96-well culture plates (1×10^4 cells/well). After 24 h incubation, cells were treated with different concentrations (0.001-1000 μM) of synthesized compounds **3a-j** for 48 h incubation, whereas in case of MOLT-4 cell line, cells were plated in 96-well culture plates (1×10^5 cells/well) and immediately treated with compounds **3a-j** for 24 h incubation. After incubation with compounds, 20 μL of MTT solution (5 mg/mL) was added to each well and incubated for 2 h, the blue MTT formazan precipitate formed was then dissolved in acidified isopropanal solution. The plates were kept at room temperature for 10 minutes on orbital shaker. The absorbance was measured in micro plate reader (TECAN infinite 200 PRO) at 570 nm. The cell viability ratio was calculated by the following formula:

$$\text{Cell survival ratio (\%)} = \frac{\text{treated} \times 100\%}{\text{OD control}}$$

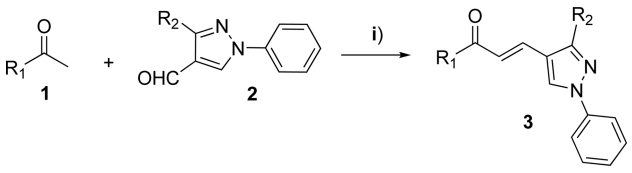
All the experiments were performed at least in triplicate, and results are summarized in terms of IC_{50} values in Table 1. In the graphs, each point corresponds to a compound concentration and is the mean of three measurements. The bars that appear in graphs are calculated from the standard error of the mean.

Intracellular Detection of Reactive Oxygen Species (ROS) by Flow Cytometery. The loss of mitochondrial membrane potential is associated with mitochondrial production of reactive oxygen species (ROS). Therefore, we determined whether ROS production is increased on treatment of the test compounds **3a-j**, Figure 2. The assay was based on previously described method.[22] The generation of ROS was measured using the 2,7-dichlorodihydrofluorescein diacetate (H_2-DCFDA) a non fluorescent dye. The acetate moiety of the dye is lost by cellular esterase leaving the non fluorescent 2,7-dichlorofluorescin (DCFH). Hydrogen peroxide and peroxidase produced by the cell oxidize DCFH to 2,7-dichlorofluorescein (DCF) which is highly fluorescent (530 nm). The green fluorescence measured is thus proportional to H_2O_2 produced. Thus, ROS production by stimulated cells causes an increase in the fluorescence signal over time. In brief, MOLT-4 cells were plated at a density of 1×10^6 cells/wells in 6-well plates and treated with 100 μM compounds (**3a-j**) or DMSO as a control for 3 h. Cells were washed with PBS. Then cells were suspended in PBS containing H_2-DCFDA (5 μM) for 20 min at 37 °C in the dark. The cells were harvested and again washed with PBS, DCF fluorescence was measured by FC 500 Beckman Coulter flow cytometer. The fluorescence intensity of treatments was compared with control. At least ten thousands events were analyzed per sample.

Among the tested compounds, **3d** displayed the highest ROS level. Thus, we can conclude that induction of apoptosis by these prop-2-en-1-one derivatives comprises the production of ROS.[23,24]

Results and Discussion

Chemistry. The compounds **3a-j** were prepared by Claisen-Schmidt condensation of appropriate commercially available ketones (**1**) with 4-formyl pyrazole derivatives (**2**) in equimolar ratio using aqueous sodium hydroxide in ethanol as outlined in Scheme 1. The aldehydes (**2**) were synthesized by Vilsmeir-Haack reaction of corresponding hydrazones, which eventually were prepared by the reaction of ketones with phenyl hydrazine according to literature procedure.[25] In IR spectra, the compounds showed a characteristic band between 1633-1647 cm^{-1} due to >C=O stretching of α, β unsaturated carbonyl group. Appearance of two doublets with coupling constant values (J = 15.7 Hz) in NMR spectra confirms the formation of trans isomeric products. The high resolution mass spectra of the compounds were in conformity with the assigned structures.



Scheme 1. Synthesis of derivatives **3a-j**. Reagents and conditions: **i**) EtOH, NaOH, 0 °C.

Table 1. Result of the cytotoxicity of the compounds (**3a-j**) against HUH7, Hep-3b and MOLT-4 cancer cell lines

Entry	R_1	R_2	IC_{50} values (μM)[a,b]		
			HUH7	Hep-3b	MOLT-4
3a	Thien-2-yl	CH_3	12.58	67.61	46.77
3b	Phenyl	CH_3	>100	3.39	89.13
3c	4-Hydroxy-6-methyl-2*H*-pyran-3-yl	CH_3	33.88	48.98	48.98
3d	5-Methylfuran-2-yl	CH_3	63.1	33.11	3.63
3e	2*H*-Chromen-3-yl	CH_3	>100	>100	>100
3f	4-Fluorophenyl	CH_3	>100	8.71	>100
3g	5-Bromofuran-2-yl	CH_3	>100	4.9	93.33
3h	4-Nitrophenyl	CH_3	97.72	>100	>100
3i	4-Fluorophenyl	Ph	>100	>100	>100
3j	5-Methylfuran-2-yl	Ph	>100	>100	51.29

[a]All the compounds were examined in a set of experiments repeated three times. [b]Values are reported as IC_{50}, the concentration of the compounds required to cause 50% inhibition of cell growth.

Biology. In order to discover novel anti-cancer agents, all the synthesized compounds **3a-j** were screened for their *in vitro* cytotoxicity against three cancer cell lines, two hepatocarcinoma cell lines (HuH-7, Hep-3b) and one leukemia cancer cell line (MOLT-4) by employing MTT colorimetric method (Mossmann T., 1983). The results of the present investigation are summarized in Table 1 and Figure 1. As shown in Table 1, the lead compounds, 1-phenyl-3-(3-methyl-1-phenyl-1*H*-pyrazole-4-yl)prop-2-en-1-one (**3b**) and 1-(5-methylfuran-2-yl)-3-(3-methyl-1-phenyl-1*H*-pyrazole-4-yl)prop-2-en-1-one (**3d**) strongly inhibits the growth of Hep-3b and MOLT-4 cells with IC_{50} value of 3.39 & 3.63 μM respectively, Figure 1(b, c). But replacing the substituents at the R^1 did not result in a significant increase in activity. Among the compounds, **3g** and **3f** with 5-bromofuran and 4-fluorophenyl at R^1 display a good level of activity holding the growth of Hep-3b with IC_{50} value of 4.9 and 8.71 μM, Table 1, Figure 1(b). Based on the above results, further simple modification in the structure did not show further enhancements in activity. However, compound **3a** with thiophene moiety at R^1 displayed moderate activity (IC_{50} = 12.58 μM). We envisioned that more potent inhibitors would be obtained by replacing the methyl group at position-3 of pyrazole in R^2 with phenyl group (**3i-j**) but none of the compounds demonstrated increase in activity. Thus, the results obtained from the present study suggested that lipophilic nature is important for such prop-2-en-1-ones to increase the antiproliferative activity on hepatic and leukemia cancer cells.

In support of the results obtained from MTT assay, compounds (**3a-j**) were subjected to measure reactive oxygen species (ROS) by flow cytometery against MOLT-4 cells. The results obtained from ROS studies further reveal that all the compounds used increase the production of ROS in leukemia cancer cells MOLT-4 as compare to untreated cells. It is quite interesting to note that compounds **3d** showed the highest ROS level (59.9%) which are in conformity with the above results attained from MTT assay in MOLT-4 cells, Figure 2.

In general, we initially examined the effect of R^2 group, particularly by taking two different substituted pyrazole nuclei and concluded that compounds having 1-phenyl-3-methyl-1*H*-pyrazol-4-yl (**3a-h**) possess an excellent level of activity as compare to the compounds having 1,3-diphenyl-1*H*-

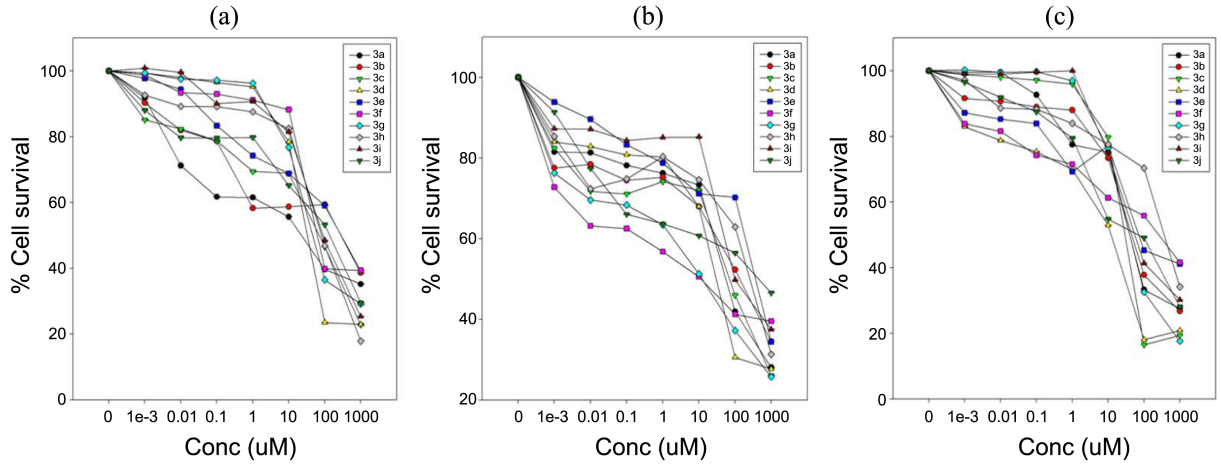


Figure 1. Anti-proliferative effects of compounds **3a-j** on HUH-7 (a), Hep-3b (b) and MOLT-4 (c) cell lines. The values for each compound concentration tested represent the average (mean ± SD) from thee replicate wells and are representative of three separate experiments.

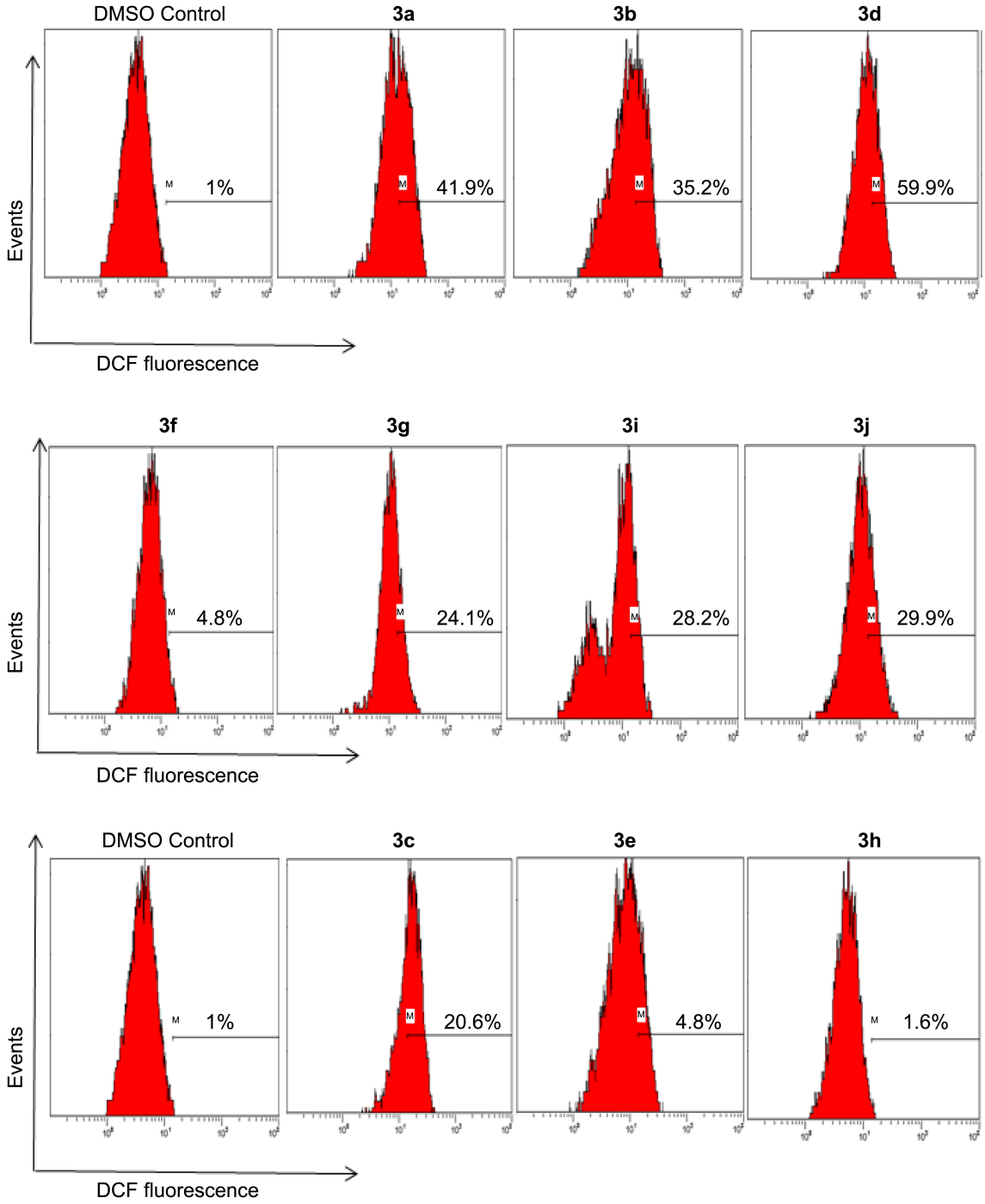


Figure 2. Intracellular generation of ROS in leukemia cancer cell line (MOLT-4) by compounds (**3a-j**). MOLT-4 cells were treated with DMSO, DMF or 100 μM chemical compounds for 3 h, cells were harvested, washed twice in PBS, re-suspended in PBS supplemented with H_2-DCFDA (5 μM). DCF fluorescence analyzed by flow cytometry. The percentage in DCF histograms indicates the MFI (mean fluorescence intensity).

pyrazol-4-yl moiety (**3i-j**). Moreover, it has also been found that compounds bearing phenyl, 4-fluorophenyl, 5-methylfuranyl and 5-bromofuranyl moieties in general exhibited excellent anticancer activity against both cancer cell lines.

Conclusion

In conclusion, we have designed and synthesized a series of some novel prop-2-en-1-one derivatives with the aim to discover novel anti-cancer agents. All the synthesized compounds were evaluated for their *in vitro* anti-cancer activities against three cancer cell lines; HUH-7, Hep-3b and MOLT-4. We conducted a structure-activity relationship (SAR) studies with the compounds to determine the optimal structure and position of the substituents for significant and specific activity. Among the tested compounds, **3b** and **3d**

has been found the most potent candidate against the hepatocarcinoma as well as MOLT-4 cell lines Hep-3b (IC_{50} = 3.39 & 3.63 μM) and brings new structural elements that will help in design of more active compounds in the future.

Furthermore, reactive oxygen species (ROS) measurement has been carried out, and compound **3d** showed the maximum ROS level, leading to apoptosis in MOLT-4 cells. These findings collectively reveal that compounds with prop-2-en-1-one framework along with pyrazole at position-3 and heteroaryl/aryl substituents at position-1 can be used as promising anti-cancer agents, deserving further investigations.

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