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Antiproliferative and tumor inhibitory studies of 2,3 disubstituted 4-thiazolidinone derivatives

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Abstract

4-thiazolidinone derivatives were synthesized using T3P®-DMSO media as a cyclodehydrating agent. All the molecules were tested for their cytotoxicity against leukemic cell lines. The compound 3-(4-bromophenyl)-2-(4-(dimethylamino)phenyl)thiazolidin-4-one (4e) with electron donating substituent at para position of phenyl ring displayed considerable cytotoxicity against Reh and Nalm6 cells with an IC_{50} value of 11.9 and 13.5 µM respectively. Furthermore, the compound 4e tested for tumor regression studies induced by EAC in Swiss albino mouse. Both in vitro and in vivo results suggested significant antiproliferative activity of compound 4e in Reh cells and mouse tumor tissue treated with compound 4e showed multifocal areas of necrosis and numerous number of apoptotic cells.

Keywords

4-thiazolidinone, T3P, Reh, EAC, Tumor regression.

Small molecule libraries have become an important source for the discovery of new drug molecules. In particular heterocycles with azolidinone ring play an important role in bio-organic and medicinal chemistry, especially reports concerning 4-thiazolidinone-containing heterocyclic compounds have gradually increased because of their pharmaceutical applications.[1,2]

4-thiazolidinone has been considered as a magic moiety (wonder nucleus) and represents an important class of heterocyclic compounds with a wide spread biological applications. Thiazolidinone derivatives have been investigated for a range of pharmacologic indications such

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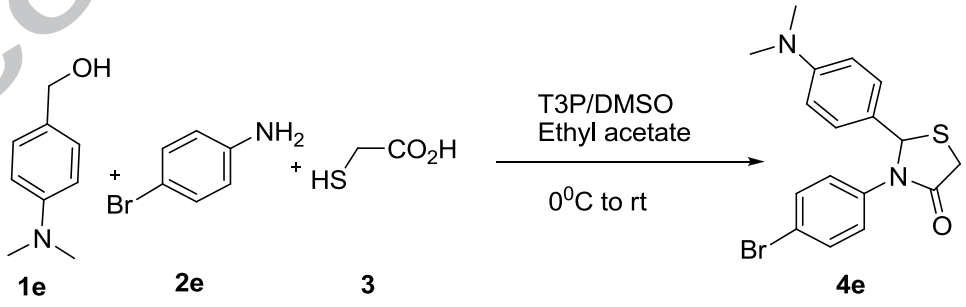
as anti-viral,[3] anti-convulsant,[4] cardiovascular,[5] anti-inflammatory,[6] antidiabetic,[7] antihyperlipidemic,[8] antimicrobial,[9] antituberculosis,[10] antiparasitis,[11] FSH agonist,[12] antiarthritic[13] and antidiarrhoeal[14] activity. In addition, 4-thiazolidinones are known to possess good activity against different forms of cancer like, breast cancer (MCF-7),[15] JNK stimulating phosphatase-1 (JSP-1),[16] tumor necrosis factor (TNFα),[17] antiapoptotic biocomplex (Bcl-XL-BH_3),[18] integrin αvβ3 receptor,[19] colon cancer (HT29)[20] and CDK1/Cyclin B inhibiton.[21] But their anti-leukemic effects have been less widely documented.[22]

From the past few years we have been engaged in the development of new synthetic routes[23] for the synthesis of various bioactive heterocyclic compounds and recently we have reported a novel propylphosphonic anhydride - DMSO mediated one pot synthesis of 4-thiazolidinone derivatives from variety of primary and secondary alcohols (Scheme 1).[24] That method provides an excellent protocol for the synthesis of 2,3 disubstituted 4-thiazolidinone derivatives in one pot operation with excellent yields.[25] In continuation of our research work towards the identification of new synthetic compounds as chemotherapeutic agents,[26] we have screened all ten 4-thiazolidinone derivatives (4a-4j) (Table 1) which were synthesized from different aryl/heteroaryl/acyclic alcohols and aryl/heteroaryl amines bearing electron donating or electron withdrawing group with excellent yields. All the synthesized compounds (4a-4j) were characterized by elemental analysis, mass, IR and NMR spectroscopy. The IR spectra show a characteristic peak at 1720-1740 cm^{-1} corresponds to carbonyl group of thiazolidinone. In further, the ^1H NMR spectra of all the compounds showed a multiplet at δ 3.83-4.05 due to the diastereotopic C_5H_2 protons of 4-thiazolidinone ring and in the ^{13}C NMR the carbonyl carbon C_4 of the thiazolidinone ring appeared at δ 170.0-171.9.



Scheme: 1 Synthesis of 3-(4-bromophenyl)-2-(4-(dimethylamino)phenyl)thiazolidin-4-one**(4e)**

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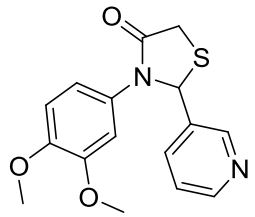
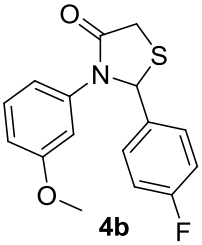
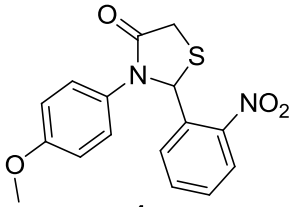
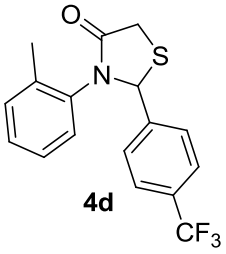
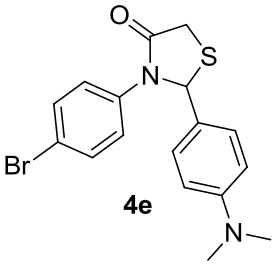
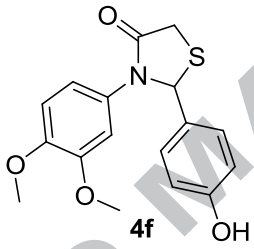
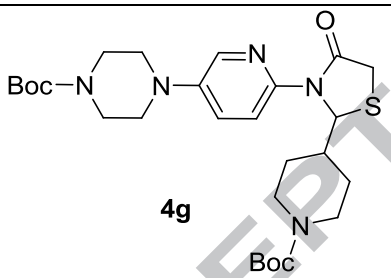
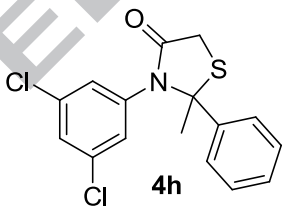
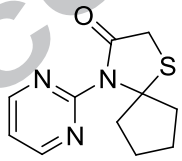
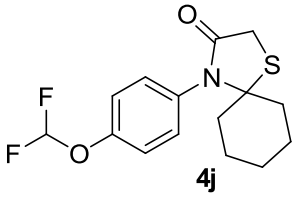
Table 1. Chemical structures of compounds 4a-4j

 **4a**	 **4b**
 **4c**	 **4d**
 **4e**	 **4f**
 **4g**	 **4h**
 **4i**	 **4j**

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Proliferation rate of Reh and Nalm6 cells in presence of different concentration of thiozolidinone derivatives (4a-4j) were tested at 48 h time point using MTT assay.[27] Results showed significant antiproliferative effect of thiozolidinone derivatives on Reh cells compared to Nalm6 cells. Importantly compound 4e showed potent antiproliferative activity on Reh cells followed by 4d, 4j and 4f. 4e exhibited IC_{50} value of 11.9 μM on Reh cells and 13.5 μM on Nalm6 cells (Table 2). Since 4e showed significant antiproliferative activity on Reh cells, it was chosen for further mechanistic studies. Significant antiproliferative activity of compound 4e indicated aberrant cell cycle distribution of compound 4e treated Reh cells. Results indicated that compound 4e was able to accumulate cells in SubG1 phase of cell cycle in concentration dependent manner (Fig. 1), suggested that, compound 4e can induce cell death in Reh cells. Decreased mitochondrial membrane potential is one of the important mechanisms for the mitochondrial mediated apoptotic cell death, therefore mitochondrial membrane potential of compound 4e treated Reh cells was investigated.[28] Interestingly, increased green fluorescence of JC-1 dye in a dose dependent manner of compound 4e treatment was observed which indicated the lowering of the mitochondrial membrane potential. Our results suggest that compound 4e could decrease mitochondrial membrane potential and induce cell death in leukemic cell line through apoptotic process (Fig. 2). The compound 4e treatment on mice bearing EAC resulted in considerable reduction in tumor volume without affecting the function of other organs (Fig. 3). Besides, the histological evaluation were showed that the morphology and cellular construction of the organs was unaltered when treated with the compound 4e. Despite existence of proliferative tumor cells, the tumor tissue microscopically revealed multifocal areas of necrosis and innumerable number of apoptotic cells (Fig. 4) following treatment with compound 4e.

In conclusion, we have found 2,3 disubstituted 4-thiazolidinone based small molecules are biologically active against human cancer cells ex vivo. In vivo data suggested tumor regression property of compound 4e in EAC tumor model. Further studies on synthesized derivatives would help to identify novel thiazolidinone compounds as potential anticancer therapeutics.

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Table 2. Antiproliferative activity of 4-thiazolidinones

Compound	Name of the cell line (IC_{50} in µM)		
	Reh	Nalm6	HEK 293T
4a	>100	>100	N.T
4b	>100	49.8	N.T
4c	>100	93.4	N.T
4d	29.6	42.6	N.T
4e	**11.9**	**13.2**	**>80**
4f	69.4	>100	N.T
4g	>100	82.9	N.T
4h	>100	>100	N.T
4i	>100	>100	N.T
4j	41.4	>100	N.T

**N.T = Not tested*

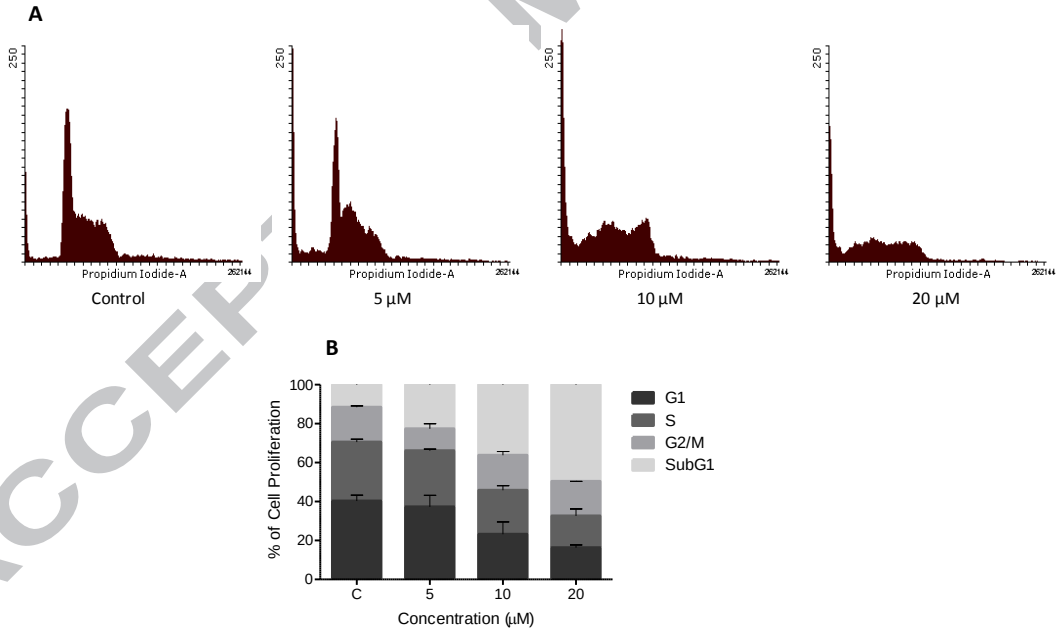

Figure 1. Cell cycle analysis of compound 4e treated Reh cells. Compound 4e treated (5, 10 and 20 µM, for 48 h) Reh cells were subjected to cell cycle analysis, DMSO treated cells were used as vehicle control. A) Histograms representing cell cycle distribution of control and compound 4e treated cells. B) Bar diagram representing percentage of cell population in different phase of the cell cycle in control and compound 4e treated Reh cells.

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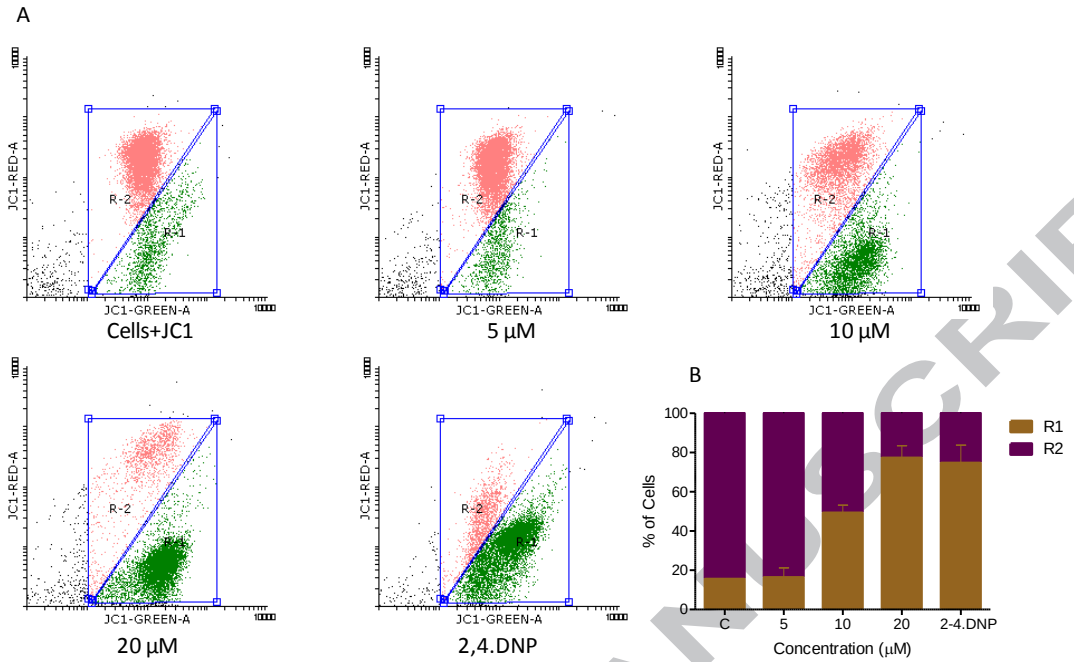

Figure 2. Analysis of mitochondrial membrane potential of compound 4e treated Reh cells. Compound 4e treated (5, 10 and 20 µM, for 48 h) Reh cells were subjected to JC-1 staining to analyze mitochondrial membrane potential. DMSO treated cells were used as control and 2, 4-DNP treated cells were used as positive control. A) Dot plots representing distribution of red and green fluorescing Reh cells in control and compound 4e treated cases. B) Bar diagram representing percentage positive cells for red (R1) and green fluorescent (R2).

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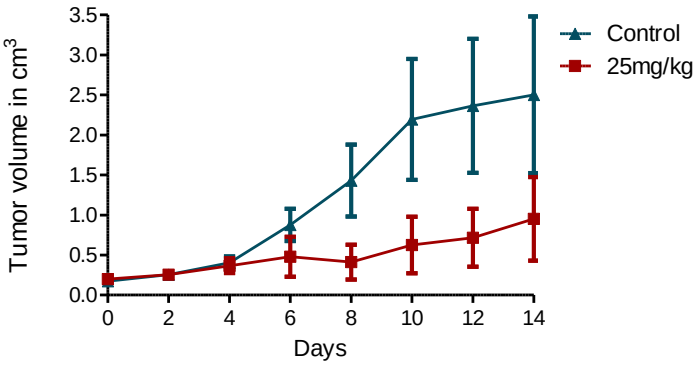

Figure 3. Tumor volume following compound 4e treatment in mice.

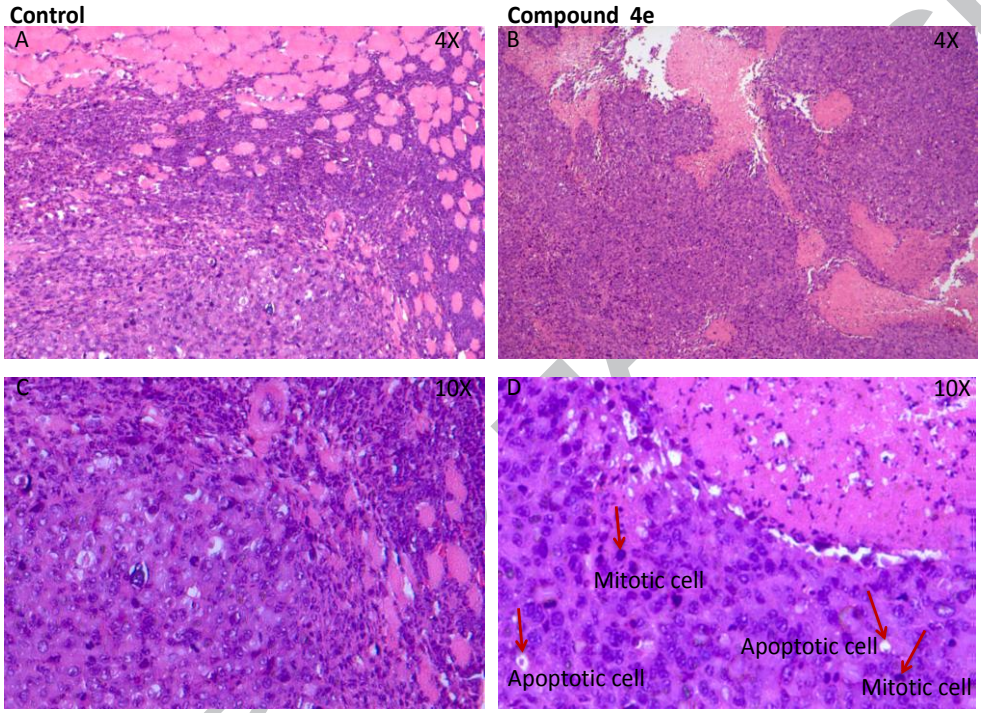

Figure 4. **A)** Section of tumor mass showing solid arrangement of neoplastic cells (4X). **C**) Section of tumor mass showing solid arrangement of neoplastic cells (10X). **B)** Section of tumor mass treated with compound 4e showing multifocal areas of necrosis and apoptotic cells (4X) **D)** Section of tumor mass treated with compound 4e showing multifocal areas of necrosis and apoptotic cells (10X).

Acknowledgments:

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25. Synthesis of 3-(3,4-dimethoxyphenyl)-2-(pyridin-3-yl)thiazolidin-4-one (4a): To a solution of 3-Pyridinemethanol 1a (1.1 mmol) in a mixture of solvents EtOAc: DMSO (4ml: 2ml), was added T3P® (2.5 mmol, 50% solution in ethyl acetate) at 0^0C, and the resulting mixture was stirred at room temperature for 1-2h under nitrogen atmosphere. 3,4-dimethoxyaniline 2a (1.0 mmol) and thioglycolic acid (1.0 mmol) were added once and stirred further for 1-3 h at room temperature. After completion of the reaction, the mixture was diluted with water (20 ml) and neutralized by adding 10% $NaHCO_3$ solution. The product was extracted with ethyl acetate (10 ml × 2) and the combined organic layers were washed with water followed by brine solution. The organic phase was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to afford a crude product which was purified by column chromatography using hexane: ethyl acetate mixture (8:2) as an eluent.
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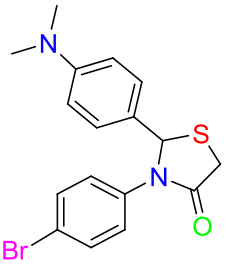

Reh IC_{50} = 11.9 μM
NALM6 IC_{50} = 13.5 μM

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