Synthesis and antiproliferative effect of novel 4-thiazolidinone-, pyridine- and piperazine-based conjugates on human leukemic cells

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Original article

Synthesis and antiproliferative effect of novel 4-thiazolidinone-, pyridine- and piperazine-based conjugates on human leukemic cells

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ABSTRACT

The present work reveals the synthesis and antiproliferative effect of a series of 2, 3 disubstituted 4-thiazolidinone analogues on human leukemic cells. The chemical structures of newly synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. Compound methyl 3-methoxy-4-(4-oxo-3-(5-(piperazin-1-yl)pyridin-2-yl)thiazolidin-2-yl)benzoate (**5**) displayed potent activity ($\mathbf{IC_{50}}$ **9.71**, **15.24** and **19.29** μM) against Nalm6, K562, Jurkat cells. Cell cycle analysis and mitochondrial membrane potential further confirmed that compound **5** is cytotoxic and able to induce cell death.

1. Introduction

Cancer is the uncontrolled proliferation and spread of cells, these proliferating cells divide rapidly (cell cycle is accelerated) and it alters cell–cell communication and leads to DNA damage. Leukemia is the most common childhood malignancy, it accounts for 30% of all cancers diagnosed in children under 15 years of age in developing countries. Therefore, there is an urgent need for new therapeutics, which could act as anti-leukemic agents with less or minimal side effects.

Heterocyclic compounds attracted a lot of attention because of its wide spread biological activities. In specific, five membered heterocycles with two heteroatoms received a special attention with proven utility in medicinal chemistry [1–3], among them 4-thiazolidinones represents an important class of five membered heterocycles. Thiazolidinones has been considered as a magic moiety (wonder nucleus) and they display diverse biological activities such as anticancer [4–6], anti-inflammatory [7], antimicrobial [8], anticonvulsant [9], antifungal [10], antitubercular [11], anti HIV [12,13] and as entamoebahistolytica inhibitors [14]. The 4-thiazolidinone moiety is very versatile and has featured in many drugs (Fig. 1) and several compounds with 4-thiazolidinone core structure were found to kill selectively drug resistant cancer cells and induce cell death [15]. Tripathi et al. [16] have given a detailed study on the synthetic approach and biological significance of 4-thiazolidinones, the review is an endeavour to highlight the progress of 4-thiazolidinone in the field of chemical biology. In addition, piperazine- and pyridine conjugated pyrimidine derivatives are well explored in the literature as selective inhibitors of CDK4/6 [17,18].

In this context, we have recently published a series of thioxothiazolidinone derivatives endowed with good activity towards the human leukemia cells [19]. Continuing our efforts in this field and considering the pharmacological activities of thiazolidinones, a new piperazine substituted pyridine conjugated 4-thiazolidinone derivatives were synthesized and screened for their antiproliferative activity against different human leukemic cell lines. Interestingly, few of the synthesized compounds showed significant improvement in the cytotoxicity profile against different leukemia cell lines in dose-dependent manner, in particular compound **5** exhibited notable cytotoxicity against Nalm6 cell line.

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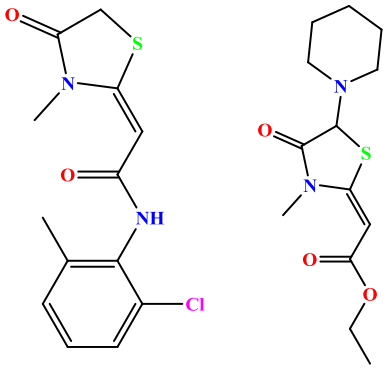


Fig. 1. Structure of lead compounds belongs to 4-thiazolidinone.

Herein, we report a mild and simple protocol for the synthesis of a library of highly functionalised 4-thiazolidinone derivatives **5**, **8a**–**f** and **9a**–**d** and their antiproliferative activities against Jurkat, K562 and Nalm6 human leukemia cell lines.

2. Results and discussion

2.1. Chemistry

In our current research, a series of novel 4-thiazolidinone-, pyridine- and piperazine based conjugates **8a**–**f** and **9a**–**d** (Table 1) bearing different level of substituents were synthesized as shown in Schemes 1 and 2. After an exhaustive exploration of the reaction conditions varying the solvents, equivalents and catalysts, optimal parameters to prepare title compounds **8a**–**f** and **9a**–**d** were found.

We have synthesized tert-butyl 4-(6-aminopyridin-3-yl)piperazine-1-carboxylate (**3**) using literature reported method [20]. Displacement of the bromine atom of 5-bromo-2-nitropyridine with 1-Boc piperazine followed by reduction of nitro group using 10% palladium on carbon in ethanol under hydrogen atmosphere afforded amino pyridine (**3**).

N-Bocpiperazine substituted 2-amino pyridine (**3**) was used as an amine component to construct 4-thiazolidinone ring. Condensation of methyl 4-formyl-3-methoxy-benzoic acid methyl ester with 2-amino pyridine (**3**) followed by cyclization with mercaptoacetic acid gave the required product tert-butyl 4-(6-(2-(2-methoxy-4-(methoxycarbonyl)phenyl)-4-oxothiazolidin-3-yl)pyridin-3-yl)piperazine-1-carboxylate (**4**) in good yield. This particular reaction is the key step in our whole reaction scheme, so we examined this reaction with different catalyst under various reaction conditions. Among all, propylphosphonic anhydride ($T_3P^{®}$) was found to be an efficient cyclodehydrating agent to construct the 4-thiazolidinone ring. This protocol is having many advantages over the literature reported methods like one pot operation, short reaction time, mild reaction condition and ease of product purification with excellent yield. It is noteworthy to mention that, the Boc group of piperazine ring was unaffected throughout the reaction. Previously we have reported the synthesis of 4-thiazolidinones using $T_3P^{®}$-DMSO media as an oxidizing as well as cyclodehydrating agent [21].

Removal of Boc protecting group was easily achieved by the treatment of **4** with aqueous HCl at room temperature. Column purification gave the key intermediate methyl 3-methoxy-4-(4-oxo-3-(5-(piperazin-1-yl)pyridin-2-yl)thiazolidin-2-yl)benzoate (**5**) in good yield of 90%.

The requisite title compounds **8a**–**f** were synthesized by the reaction of **5** with different benzene sulfonyl chlorides **6a**–**f** in presence of triethylamine in dichloromethane solvent. In similar reaction condition the other series **9a**–**d** were synthesized by reacting **5** with different benzyl chlorides **7a**–**d** in good yields.

2.2. Biological studies

2.2.1. Piperazine substituted pyridine conjugated 4-thiazolidinones (*5*, *8a*–*f* and *9a*–*d*) induces cytotoxicity on leukemic cell lines

Inhibition of cell proliferation is a key factor for chemotherapeutic agents. In the present study we used trypan blue and MTT assay to investigate the effect of piperazine substituted pyridine conjugated 4-thiazolidinones (**5**, **8a**–**f** and **9a**–**d**) induced cytotoxicity on three different leukemic cell lines (Jurkat, K562 and Nalm6) (Fig. 2), following treatment with different concentrations of the compound (Table 2). The trypan blue dye exclusion assay showed significant reduction in cell viability compared to control upon treatment with **5**. Besides, MTT assay also showed significant anti proliferation activity, which was comparable with trypan blue assay. Out of the three cell lines tested, the Nalm6 cell line was more sensitive to the experimental compound **5**, compared to Jurkat and K562 cells, and was selected for further studies.

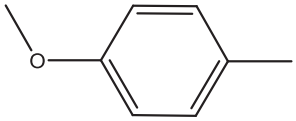
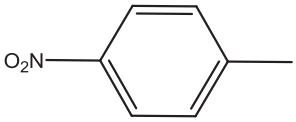
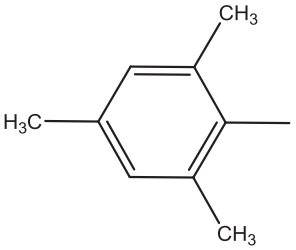
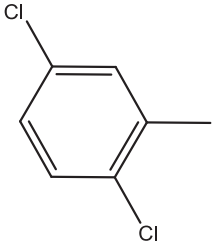
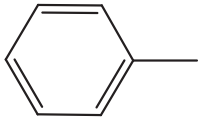
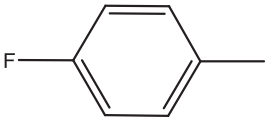
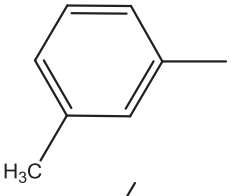
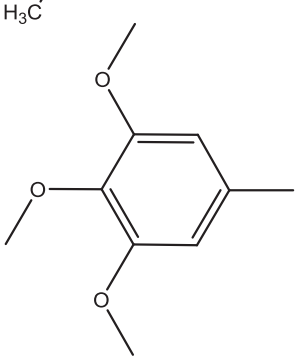
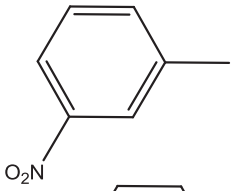
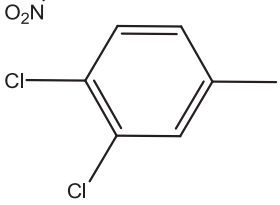
2.2.2. Cell cycle analysis following treatment with compound *5* on Nalm6 cells

After preliminary investigations of trypan blue dye exclusion and MTT assays, we tested the effect of **5** on cell cycle progression by using fluorescence activated cell sorter (FACS). Nalm6 cells were harvested after 48 h of compound **5** treatment (5, 10 and 20 μM). Cells were processed, stained with propidium iodide and subjected to flowcytometric analysis. The histogram of control (DMSO treated) cells showed standard pattern of cell cycle progression. Upon addition of **5** to Nalm6 cells, a concentration dependent change was observed in the cell cycle distribution. However, such change was minimal when the cells were treated with 5, 10 μM of compound **5**. We noted that there was remarkable accumulation of SubG1 cell population at 20 μM as well as G2/M arrest (Fig. 3). These results suggested that compound **5** arrests the cells at G2/M phase and induces cell death which was resulted in increased level of SubG1 phase population, thereby affecting its proliferation.

2.2.3. Piperazine substituted pyridine conjugated 4-thiazolidinone (*5*) induces cell death by affecting mitochondrial membrane potential

To determine the mechanism by which compound **5** induces cell death, JC-1 staining assay was performed. Effect of compound **5** on mitochondrial membrane potential was determined by JC-1 staining followed by flow cytometry. Changes in the mitochondrial membrane potential was determined by red verses green fluorescence by JC-1 dye where healthy mitochondria gives out red fluorescence because of J-aggregates of JC-1 dye and apoptotic/dead cells show green fluorescence because of lack of mitochondrial membrane potential. Panels representative of flowcytometric density plots were used to determine the results which are depicted in (Fig. 4), where cells exposed to the mitochondrial stressor 2, 4-DNP was used as positive control. DMSO treated cells served as the vehicle control. Minimum 10,000 events were acquired and analyzed by using Cell Quest Pro software (BD, USA). Results showed that compound **5** induced a significant depolarization of mitochondrial membrane potential in a dose-dependent manner on leukemic cells.

Table 1
Derivatives of 4-thiazolidinone **8a**–**f** and **9a**–**d**.

Entry	Compound	*R*	Yield (%)[a]
1	**8a**		88
2	**8b**		78
3	**8c**		82
4	**8d**		77
5	**8e**		87
6	**8f**		78
7	**9a**		74
8	**9b**		71
9	**9c**		70
10	**9d**		73

[a] Isolated yield based on intermediate **5**.

3. Conclusion

In summary, the cytotoxicity assays were used for preliminary screening of 4-thiazolidinone-, pyridine- and piperazine-based conjugates. Among all the screened derivatives, compound **5** was found to be most potent against leukemia cell lines at lower concentrations. Further we carried out several methods to elucidate the mechanism by which the compound to induced the cell death. In Nalm6 cells, compound **5** induced cell cycle arrest and caused depolarization of mitochondrial membrane potential. Hence the methyl 3-methoxy-4-(4-oxo-3-(5-(piperazin-1-yl)pyridine-2-yl) thiazolidin-2-yl)benzoate (**5**) may represent a promising new alternative for an antileukemic agent.

4. Experimental section

4.1. Chemistry

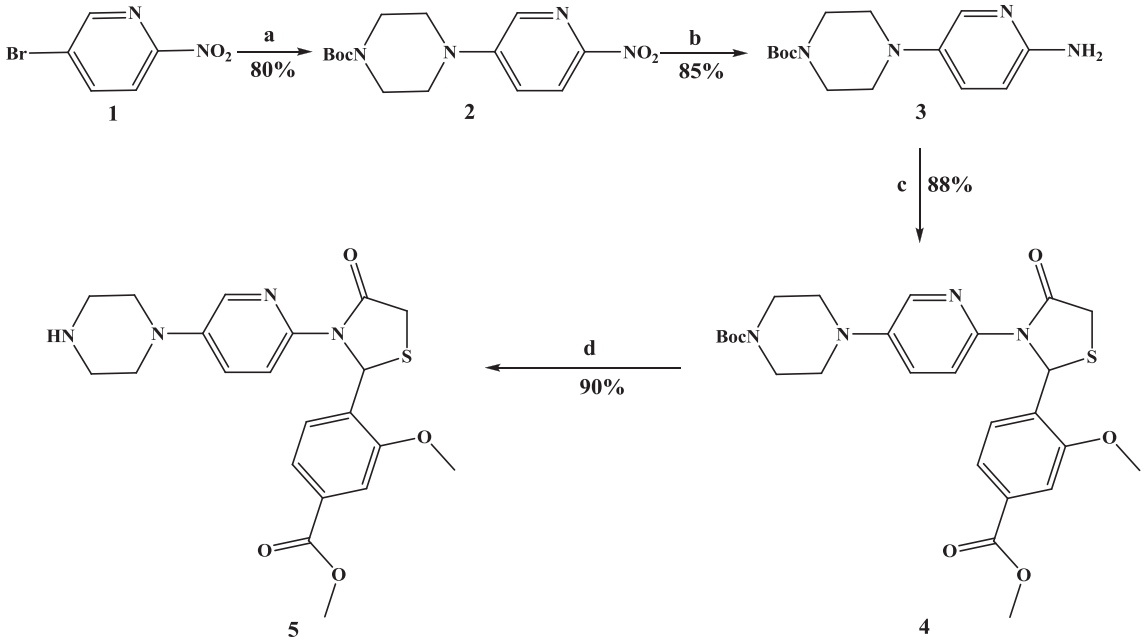
The progress of all reactions was monitored by TLC, which was performed on 2.0–5.0 cm aluminum sheets precoated with silica gel 60 F 254 to a thickness of 0.25 mm (Merck) using UV light for visualization. ^{1}H NMR and ^{13}C NMR spectra were recorded on an Agilent-varian NMR spectrometer operating at 400 and 100 MHz, respectively. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard. Mass spectra were recorded using high resolution mass spectrometer (ESI). Infrared spectra were recorded in nujol/KBr on Shimadzu FT-IR model 8300 spectrophotometer. Chromatographic separations were carried out on 60:120 silica gel.

4.1.1. Tert-butyl 4-(6-nitropyridin-3-yl)piperazine-1-carboxylate (**2**)

A solution of 1-Boc-piperazine (15.0 mmol) and 5-bromo-2-nitropyridine (5.00 mmol) in N-methyl pyrrolidone (15 mL) was stirred at 120 °C for 3 h. The reaction mixture was diluted with water and the precipitate was collected by filtration to give **2** (80%) as a colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10–8.08 (d, J = 9.2 Hz, 1H, ArH), 7.96–7.95 (d, J = 3.0 Hz, 1H, ArH), 7.33–7.30 (dd, J = 3.4 Hz, 1H, ArH), 3.66–3.63 (m, 4H, piperazine-CH_2), 3.43–3.41 (m, 4H, piperazine-CH_2), 1.47 (s, 9H, *t*-butyl-CH_3).

4.1.2. Tert-butyl 4-(6-aminopyridin-3-yl)piperazine-1-carboxylate (**3**)

A suspension of **2** and 10% palladium on carbon in ethanol was hydrogenated for 2 h. The reaction mixture was filtered through

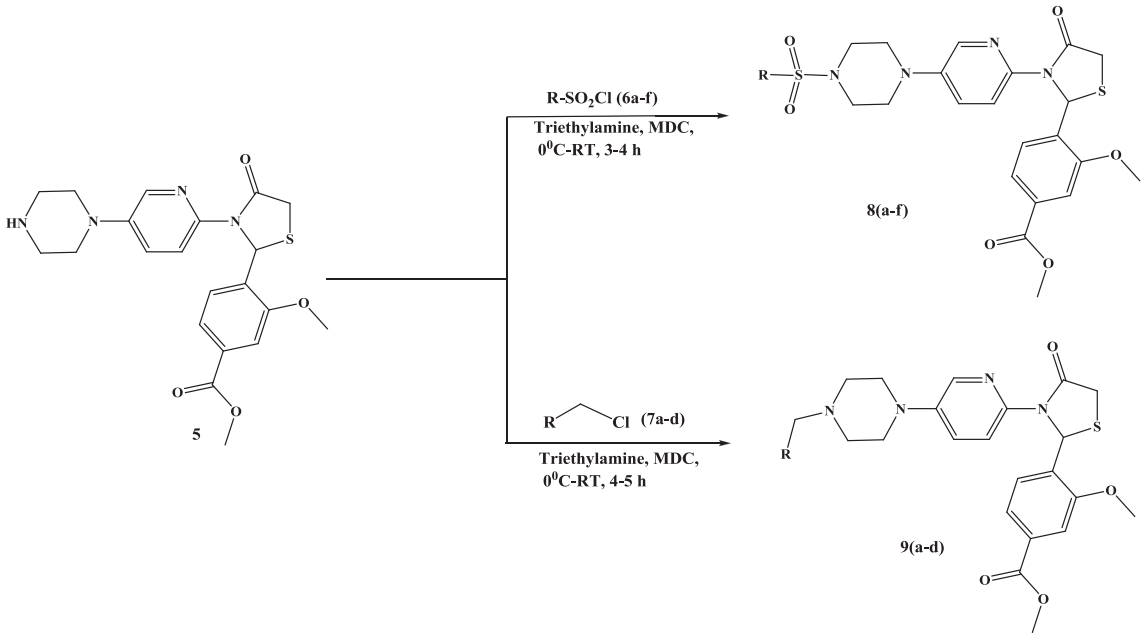


Scheme 1. Reagents and conditions: (a) 1-Boc-piperazine, N-methyl pyrrolidone, 120 °C, 3 h; (b) 10% palladium on carbon, ethanol, H_2, 2 h; (c) 4-formyl-3-methoxy-benzoic acid methyl ester, Mercaptoacetic acid, $T_3P^®$, EtOAc, 0 °C-RT, 3 h; (d) Dioxane, 4 M HCl, RT, 2 h.

celite and concentrated in vacuo to give **3** as brown oil. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.77–7.76 (d, J = 2.1 Hz, 1H, ArH), 7.27–7.14 (dd, J = 3.0 Hz, 1H, ArH), 6.51 (d, J = 9.0 Hz, 1H, ArH), 3.61–3.59 (m, 4H, piperazine-CH_2), 3.39–3.36 (m, 4H, piperazine-CH_2), 1.48 (s, 9H. *t*-butyl-CH_3).

*4.1.3. Tert-butyl 4-(6-(2-(2-methoxy-4-(methoxycarbonyl)phenyl)-4-oxothiazolidin-3-yl)pyridin-3-yl)piperazine-1-carboxylate (**4**)*

4-formyl-3-methoxy-benzoic acid methyl ester (1.0 mmol), amine **3** (1.0 mmol), thioglycolic acid (1.0 mmol) were stirred in ethyl acetate, propylphosphonic anhydride ($T_3P^®$) (1.5 mmol) was added to the reaction mixture at 0 °C. The reaction kept for stirring at room temperature for 3 h. 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 dried under reduced pressure to afford a desired product as off white solid which was pure enough to perform the next reaction. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.90–7.89 (d, J = 2.4 Hz, 1H, ArH), 7.75–7.72 (m, 2H, ArH), 7.46–7.42 (dd, J = 2.8 Hz, 1H, ArH), 7.34–7.30 (dd, J = 3.2 Hz, 1H,



Scheme 2. Synthesis of title compounds.

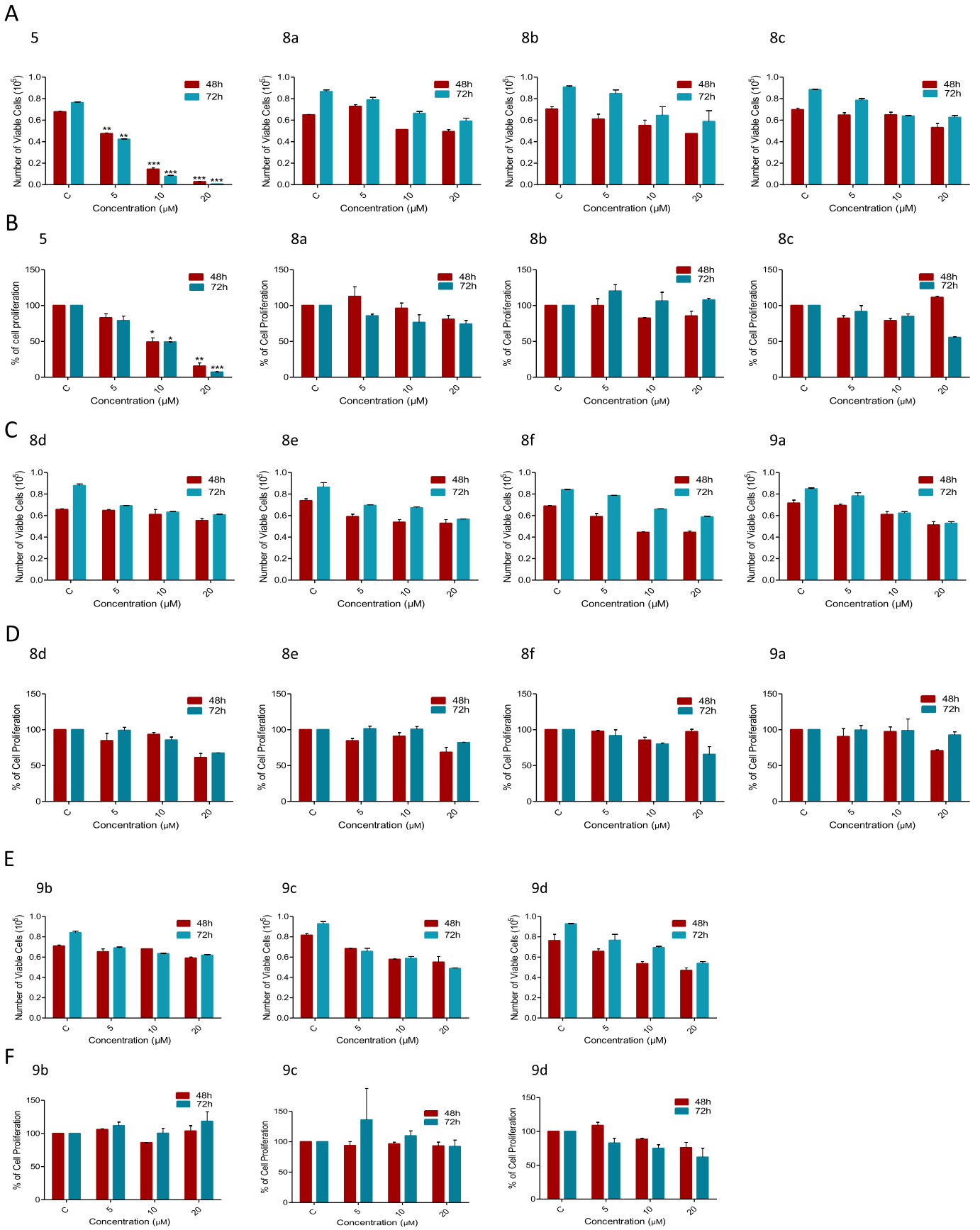


Fig. 2. Evaluation of cytotoxicity following addition of (**5**, **8a**–**f** and **9a**–**9d**) on Nalm6 cells. A, C and E shows trypan blue assay for compounds **5**, **8a**–**f** and **9a**–**9d** respectively, after 48 h and 72 h of treatment (5, 10, 20 μM). B, D and F MTT assay after addition of **5**, **8a**–**f** and **9a**–**9d**. In all panels "C" stands for DMSO treated vehicle control.

Table 2
The antiproliferative effects of thiozalidinone-, pyridine- and piperazine-based conjugates (**5**, **8a**–**f** and **9a**–**f**) in Jurkat, K562 and Nalm6 cells.

Compound	Cell line (IC_{50} in μM)		
	Jurkat	K562	Nalm6
5	19.29	15.24	9.71
8a	45.24	54.52	29.40
8b	53.96	65.21	>100
8c	>100	53.54	>100
8d	>100	>100	27.39
8e	52.92	65.58	48.44
8f	78.26	>100	>100
9a	>100	>100	27.34
9b	40.96	>100	>100
9c	37.44	68.69	>100
9d	60.99	>100	>100
Paclitaxel	0.0045	0.0055	0.0041

ArH), 6.99–6.97 (d, J = 8.2 Hz, 1H, ArH), 6.72 (s, 1H, thiazolidinone-CH), 3.96-3.90 (m, 2H, thiazolidinone-CH_2), 3.71 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.61–3.58 (m, 4H, piperazine-CH_2), 3.27–3.25 (m, 4H, piperazine-CH_2), 1.51 (s, 9H, *t*-butyl-CH_3).

*4.1.4. Methyl 3-methoxy-4-(4-oxo-3-(5-(piperazin-1-yl)pyridin-2-yl)thiazolidin-2-yl)benzoate (**5**)*

Compound **4** was stirred at ambient temperature in dioxane and 4 M HCl for 2 h. The solvent was evaporated in vacuum, the product was precipitated by adding diethyl ether. The precipitate was collected by filtration and neutralized with 10% $NaHCO_3$ solution and extracted with ethylacetate (2 times). The combined organic layer was washed with water followed by brine solution and dried over anhydrous Na_2SO_4, organic solvent was evaporated under reduced pressure to afford the crude product **5**, which was purified by silica-gel column chromatography using chloroform and methanol (1:1) as an eluent to give compound **5** as a pale-yellow solid. IR γmax (KBr) 3280, 3110, 2901, 1820, 1748, 1740, 1681, 1510, 1488, 1440, 1246, 1001 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.89–7.88 (d, J = 2.8 Hz, 1H, ArH), 7.76–7.70 (m, 2H, ArH), 7.44–7.39 (dd, J = 2.4 Hz, 1H, ArH), 7.33–7.30 (dd, J = 3.4 Hz, 1H, ArH), 6.95–6.93 (d, J = 8.8 Hz, 1H, ArH), 6.70 (s, 1H, thiazolidinone-CH), 4.02–3.90 (m, 2H, thiazolidinone-CH_2), 3.73 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.65–3.62 (m, 4H, piperazine-CH_2), 3.27–3.25 (m, 4H, piperazine-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.7, 166.1, 157.5, 142.9, 136.0, 134.4, 132.5, 131.6, 130.1, 125.0, 120.1, 118.5, 112.1, 80.0, 62.5, 56.0, 52.0, 48.6, 34.0 cm^{-1}; HRMS (ESI) *m/z* Calcd for $C_{21}H_{24}N_4O_4SNa$ $[M+Na]^+$ 451.5047, found 451.5057.

*4.1.5. General procedure for the synthesis of compounds 8(**a**–**f**)*

To a solution of methyl 3-methoxy-4-(4-oxo-3-(5-(piperazin-1-yl)pyridin-2-yl)thiazolidin-2-yl)benzoate **5** (1.00 mmol) and triethylamine (1.5 mmol) in dry dichloromethane at 0 °C were added substituted benzene sulfonyl chlorides **6a**–**f** (1.00 mmol). The reaction mixture was stirred at 0 °C for about 30 min and the stirring was continued at room temperature for about 3–4 h (completion of the reaction was monitored by TLC). After completion of the reaction, the reaction mixture was diluted with water and extracted with dichloromethane (2 times), the combined organic layer was washed with water followed by brine solution and dried over anhydrous Na_2SO_4. The solvent was dried under reduced pressure to afford a desired product.

*4.1.5.1. Methyl 3-methoxy-4-(3-(5-(4-(4-methoxyphenylsulfonyl) piperazin-1-yl)pyridin-2-yl)-4-oxothiazolidin-2-yl)benzoate (**8a**).* Yield: 88%; Pale yellow liquid; IR γmax (nujol) 3045, 1742, 1651, 1582, 1449, 1381, 1369, 1320, 1245, 1165, 1065 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 8.00–7.98 (d, J = 9.0 Hz, 1H, ArH), 7.84–7.82 (d, J = 8.2 Hz, 2H, ArH), 7.78–7.77 (d, J = 3.2 Hz, 1H, ArH), 7.58–7.57 (d, J = 2.2 Hz, 1H, ArH), 7.45–7.43 (dd, J = 2.4 Hz, 1H, ArH), 7.37–7.34 (dd, J = 3.2 Hz, 1H, ArH), 7.12–7.10 (d, J = 9.0 Hz, 1H, ArH), 7.06–7.04 (d, J = 8.2 Hz, 2H, ArH), 6.64 (s, 1H, thiazolidinone-CH), 4.03–3.99 (dd, J = 2.2 Hz, 1H, thiazolidinone-$CH_2(H_A)$), 3.83–3.79

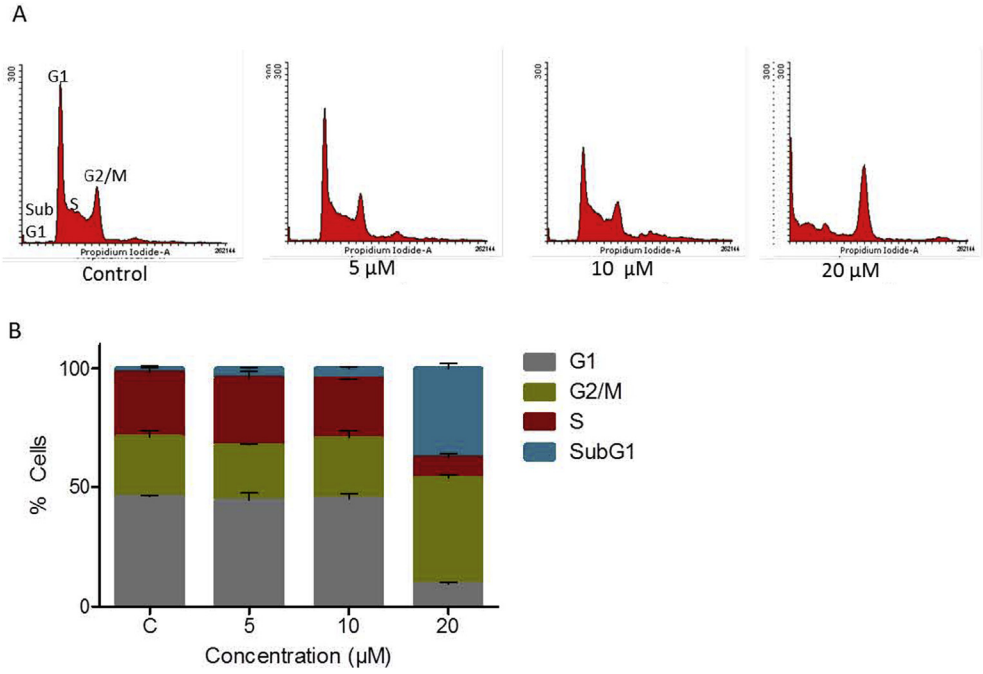


Fig. 3. Cell cycle analysis of Nalm6 cells following 5 treatment: Nalm6 cells treated with 5 (5, 10 and 20 μM) for 48 h, harvested and stained with propidium iodide and subjected to flow cytometry. (A) Histogram obtained after FACS analysis for Nalm6 cells. (B) Bar graph shows the percentage of cells in the subG1, G1, S and G2/M phases of the cell cycle. The data presented is derived from two independent experiments and error bars indicated. For each samples 10,000 cells were used for cell sorting.

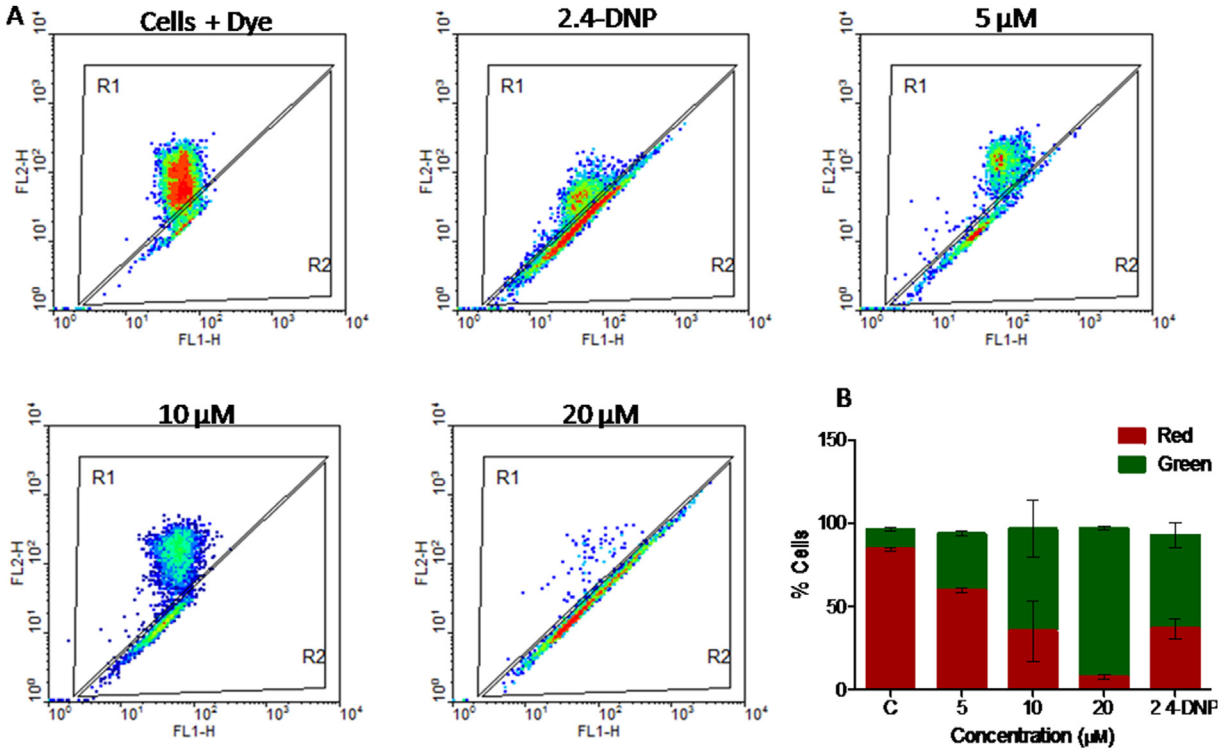


Fig. 4. Detection of loss of mitochondrial membrane potential followed by **5** treatment. Nalm6 cells treated with **5** (5, 10 and 20 μM) for 48 h, harvested and stained with JC-1 dye and analyzed by flow cytometry. 2, 4-DNP treated Nalm6 cells were served as positive control. (A) Density plot representing JC-1 stained cells at different concentration of 5. (B) Bar diagram showing ratio of red versus green fluorescent cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(m, 1H, thiazolidinone-$CH_2(H_B)$), 3.89 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.29–3.25 (m, 4H, piperazine-CH_2), 2.98–2.95 (m, 4H, piperazine-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.1, 166.3, 160.1, 158.2, 144.7, 143.1, 136.2, 134.6, 132.4, 131.8, 131.5, 130.1, 126.3, 125.2, 120.1, 118.3, 114.2, 112.2, 62.4, 56.1, 55.8, 52.1, 48.6, 33.8. HRMS (ESI) *m/z* Calcd for $C_{28}H_{30}N_4O_7S_2Na$ $[M+Na]^+$ 621.6904, found 621.6915.

*4.1.5.2. Methyl 3-methoxy-4-(3-(5-(4-(4-nitrophenylsulfonyl)piperazin-1-yl)pyridin-2-yl)-4-oxothiazolidin-2-yl)benzoate (**8b**).* Yield: 78%; Dark brown liquid; IR γmax (nujol) 3040, 1740, 1668, 1587, 1535, 1449, 1385, 1370, 1350, 1242, 1165, 1060 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.42–8.39 (m, 2H, ArH), 8.02–7.99 (m, 2H, ArH), 7.919–7.911 (d, *J* = 3.2 Hz, 1H, ArH), 7.65–7.63 (d, *J* = 9.2 Hz, 1H, ArH), 7.575–7.570 (d, *J* = 2.0 Hz, 1H, ArH), 7.46–7.43 (dd, *J* = 2.4 Hz, 1H, ArH), 7.36–7.33 (dd, *J* = 3.2 Hz, 1H, ArH), 7.00–6.98 (d, *J* = 9.2 Hz, 1H, ArH), 6.67 (s, 1H, thiazolidinone-CH) 4.04-4.00 (dd, *J* = 1.2 Hz, 1H, thiazolidinone-$CH_2(H_A)$), 3.83–3.78 (m, 1H, thiazolidinone-$CH_2(H_B)$), 3.72 (s, 6H, OCH_3), 3.21–3.19 (m, 4H, piperazine-CH_2), 3.05–3.03 (m, 4H, piperazine-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.9, 166.3, 158.0, 150.6, 144.2, 142.8, 140.9, 135.8, 133.4, 131.5, 129.5, 129.0, 125.2, 120.3, 118.4, 113.1, 61.8, 56.3, 52.4, 47.5, 45.9, 33.3. HRMS (ESI) *m/z* Calcd for $C_{27}H_{27}N_5O_8S_2Na$ $[M+Na]^+$ 636.6620, found 636.6637.

*4.1.5.3. Methyl 4-(3-(5-(4-(mesitylsulfonyl)piperazin-1-yl)pyridin-2-yl)-4-oxothiazolidin-2-yl)-3-methoxybenzoate (**8c**).* Yield: 82%; Pale yellow oil; IR γmax (nujol) 3054, 2987, 1742, 1669, 1582, 1448, 1383, 1372, 1251, 1168, 1058 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.92–7.91 (d, *J* = 3.0 Hz, 1H, ArH), 7.66–7.64 (d, *J* = 9.0 Hz, 1H, ArH), 7.58-7.57 (d, *J* = 2.4 Hz, 1H, ArH), 7.45–7.42 (dd, 2.4 Hz, 1H, ArH), 7.35–7.32 (dd, *J* = 3.2 Hz, 1H, ArH), 7.21 (s, 2H), 6.94–6.92 (d, *J* = 9.0 Hz, 1H, ArH), 6.69 (s, 1H, thiazolidinone-CH), 4.03–4.00 (dd, *J* = 1.4 Hz, 1H, thiazolidinone-$CH_2(H_A)$), 3.83–3.81 (m, 1H, thiazolidinone-$CH_2(H_B)$), 3.73 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 3.18–3.16 (m, 4H, piperazine-CH_2), 3.03–3.00 (m, 4H, piperazine-CH_2). 2.62 (s, 6H, CH_3), 2.58 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.0, 166.2, 158.8, 145.2, 144.6, 142.8, 141.2, 137.3, 136.0, 134.5, 132.4, 131.7, 130.3, 130.1, 125.2, 120.0, 118.9, 112.2, 61.9, 56.2, 52.2, 48.9, 34.2, 22.5, 22.0. HRMS (ESI) *m/z* Calcd for $C_{30}H_{34}N_4O_6S_2Na$ $[M+Na]^+$ 633.7442, found 633.7451.

*4.1.5.4. Methyl 4-(3-(5-(4-(2,5-dichlorophenylsulfonyl)piperazin-1-yl)pyridin-2-yl)-4-oxothiazolidin-2-yl)-3-methoxybenzoate (**8d**).* Yield: 77%; Dark brown gummy solid; IR γmax (nujol) 3050, 1737, 1658, 1576, 1450, 1380, 1367, 1240, 1163, 1062, 730 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.01–7.99 (d, *J* = 9.2 Hz, 1H, ArH), 7.89–7.88 (d, *J* = 3.2 Hz, 1H, ArH), 7.84–7.83 (d, *J* = 2.8 Hz, 1H, ArH), 7.70–7.68 (d, *J* = 6.8 Hz, 2H, ArH), 7.58–7.57 (d, *J* = 2.4 Hz, 1H, ArH), 7.44–7.41 (dd, *J* = 2.2 Hz, 1H, ArH), 7.35–7.32 (dd, *J* = 3.4 Hz, 1H, ArH), 7.01–6.99 (d, *J* = 9.0 Hz, 1H, ArH), 6.72 (s, 1H, thiazolidinone-CH), 4.04–4.00 (dd, *J* = 1.2 Hz, 1H, thiazolidinone-$CH_2(H_A)$), 3.85–3.82 (m, 1H, thiazolidinone-$CH_2(H_B)$), 3.74 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.27–3.24 (m, 4H, piperazine-CH_2), 3.07–3.05 (m, 4H, piperazine-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.9, 166.0, 159.1, 144.9, 142.7, 141.4, 135.9, 134.5, 133.3, 132.9, 132.5, 131.6, 130.6, 130.1, 129.7, 125.0, 119.9, 118.3, 112.2, 62.4, 56.2, 52.1, 48.7, 34.1. HRMS (ESI) *m/z* Calcd for $C_{27}H_{26}Cl_2N_4O_6S_2Na$ $[M+Na]^+$ 660.5545, found 660.5553.

*4.1.5.5. Methyl 3-methoxy-4-(4-oxo-3-(5-(4-(phenylsulfonyl)piperazin-1-yl)pyridin-2-yl)thiazolidin-2-yl)benzoate (**8e**).* Yield: 87%; Yellow gummy liquid; IR γmax (nujol) 3050, 1738, 1650, 1575, 1450,

1375, 1365, 1240, 1162, 1066 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.90–7.89 (d, J = 2.8 Hz, 1H, ArH), 7.74–7.69 (m, 3H, ArH), 7.65–7.61 (m, 3H, ArH), 7.59–7.58 (d, J = 2.4 Hz, 1H, ArH), 7.46–7.44 (dd, J = 2.4 Hz, 1H, ArH), 7.34–7.31 (dd, J = 3.2 Hz, 1H, ArH), 7.00–6.98 (d, J = 9.2 Hz, 1H, ArH), 6.68 (s, 1H, thiazolidinone-CH), 4.05–4.00 (dd, J = 1.2 Hz, 1H, thiazolidinone-$CH_2(H_A)$), 3.83–3.79 (m, 1H, thiazolidinone-$CH_2(H_B)$), 3.724 (s, 3H, OCH_3), 3.720 (s, 3H, OCH_3), 3.17–3.15 (m, 4H, piperazine-CH_2), 2.94–2.91 (m, 4H, piperazine-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.9, 166.2, 158.0, 144.2, 142.8, 135.8, 134.8, 133.8, 133.4, 131.5, 129.9, 129.0, 128.0, 125.2, 120.3, 118.4, 113.1, 61.8, 56.2, 52.4, 47.5, 46.0, 33.3. HRMS (ESI) m/z Calcd for $C_{27}H_{28}N_4O_6S_2Na$ $[M+Na]^+$ 591.6644, found 591.6652.

*4.1.5.6. Methyl 4-(3-(5-(4-(4-fluorophenylsulfonyl)piperazin-1-yl) pyridin-2-yl)-4-oxothiazolidin-2-yl)-3-methoxybenzoate (**8f**).* Yield: 78%; Pale brown liquid; IR γmax (nujol) 3043, 1739, 1648, 1588, 1448, 1385, 1371, 1245, 1163, 1060, 1090 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.92–7.91 (d, J = 2.6 Hz, 1H, ArH), 7.86–7.84 (d, J = 7.8 Hz, 2H, ArH), 7.82–7.81 (d, J = 3.2 Hz, 1H, ArH), 7.61–7.60 (d, J = 2.4 Hz, 1H, ArH), 7.46–7.43 (dd, J = 2.8 Hz, 1H, ArH), 7.39–7.36 (dd, J = 3.2 Hz, 1H, ArH), 7.34–7.32 (d, J = 7.8 Hz, 2H, ArH), 7.00–6.98 (d, J = 8.8 Hz, 1H, ArH), 6.71 (s, 1H, thiazolidinone-CH), 4.00–3.95 (dd, J = 1.2 Hz, 1H, thiazolidinone-$CH_2(H_A)$), 3.86–3.83 (m, 1H, thiazolidinone-$CH_2(H_B)$), 3.75 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.19–3.16 (m, 4H, piperazine-CH_2), 2.93–2.90 (m, 4H, piperazine-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.9, 166.6, 166.1, 158.8, 144.9, 143.1, 136.2, 135.3, 134.4, 132.6, 131.8, 130.7, 130.1, 125.2, 120.4, 118.7, 115.4, 112.2, 62.8, 56.2, 51.8, 48.4, 33.2. HRMS (ESI) m/z Calcd for $C_{27}H_{27}FN_4O_6S_2Na$ $[M+Na]^+$ 609.6549, found 609.6558.

*4.1.6. General procedure for the synthesis of compounds **9(a–d)***

To a solution of methyl 3-methoxy-4-(4-oxo-3-(5-(piperazin-1-yl) pyridin-2-yl)thiazolidin-2-yl)benzoate **5** (1.00 mmol) and triethylamine (1.5 mmol) in dry dichloromethane at 0 °C were added substituted benzyl chlorides **7a–d** (1.00 mmol). The reaction mixture was stirred at 0 °C for about 30 min and the stirring was continued at room temperature for about 4–5 h (completion of the reaction was monitored by TLC). After completion of the reaction, the reaction was diluted with water and extracted with dichloromethane (2 times), the combined organic layer was washed with water followed by brine solution and dried over anhydrous Na_2SO_4. The solvent was dried under reduced pressure to afford a desired product.

*4.1.6.1. Methyl 3-methoxy-4-(3-(5-(4-(3-methylbenzyl)piperazin-1-yl)pyridin-2-yl)-4-oxothiazolidin-2-yl)benzoate (**9a**).* Yield: 74%; Yellow liquid; IR γmax (nujol) 3057, 1735, 1658, 1579, 1445, 1383, 1369, 1240, 1167, 1061 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.90–7.89 (d, J = 2.6 Hz, 1H, ArH), 7.66–7.64 (d, J = 9.2 Hz, 1H, ArH), 7.59–7.58 (d, J = 2.2 Hz, 1H, ArH), 7.46–7.43 (dd, J = 2.4 Hz, 1H, ArH), 7.41–7.38 (m, 1H, ArH), 7.34–7.30 (dd, J = 3.2 Hz, 1H, ArH), 7.19 (s, 1H, ArH), 7.10–7.08 (d, J = 9.0 Hz, 1H, ArH), 7.06–7.01 (m, 2H, ArH), 6.68 (s, 1H, thiazolidinone-CH), 4.04–4.01 (dd, J = 1.2 Hz, 1H, thiazolidinone-$CH_2(H_A)$), 3.86–3.83 (m, 1H, thiazolidinone-$CH_2(H_B)$), 3.74 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 3.63 (s, 2H, benzyl-CH_2), 3.21–3.19 (m, piperazine-CH_2), 3.05–3.03 (m, piperazine-CH_2), 2.29 (s, 3H, Ar–CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.8, 166.1, 158.8, 144.9, 142.7, 138.8, 138.2, 136.2, 134.6, 132.2, 131.4, 130.9, 130.1, 128.2, 127.1, 125.7, 125.1, 120.3, 118.9, 112.4, 64.2, 62.6, 56.2, 52.1, 48.9, 33.4, 21.2. HRMS (ESI) m/z Calcd for $C_{29}H_{32}N_4O_4SNa$ $[M+Na]^+$ 555.6538, found 555.6547.

*4.1.6.2. Methyl 3-methoxy-4-(4-oxo-3-(5-(4-(3,4,5-trimethoxybenzyl)piperazin-1-yl)pyridin-2-yl)thiazolidin-2-yl)benzoate (**9b**).* Yield: 71%; Brown gummy solid; IR γmax (nujol) 3049, 1737, 1658, 1586, 1451, 1392, 1369, 1249, 1176, 1058 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.88–7.87 (d, J = 2.8 Hz, 1H, ArH), 7.66–7.64 (d, J = 9.2 Hz, 1H, ArH), 7.57–7.56 (d, J = 2.4 Hz, 1H, ArH), 7.44–7.41 (dd, J = 2.2 Hz, 1H, ArH), 7.38–7.35 (dd, J = 3.0 Hz, 1H, ArH), 7.02–7.00 (d, J = 8.8 Hz, 1H, ArH), 6.76 (s, 2H, ArH), 6.66 (s, 1H, thiazolidinone-CH), 4.06–4.03 (dd, J = 1.2 Hz, 1H, thiazolidinone-$CH_2(H_A)$), 3.88–3.85 (m, 1H, thiazolidinone-$CH_2(H_B)$), 3.76 (s, 3H, OCH_3), 3.73 (s, 12H, OCH_3), 3.64 (s, 2H, benzyl-CH_2), 3.26–3.23 (m, 4H, piperazine-CH_2), 3.04–3.02 (m, 4H, piperazine-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.7, 166.2, 158.7, 152.1, 144.6, 142.6, 137.7, 136.0, 134.1, 132.6, 131.8, 130.3, 129.6, 125.1, 120.2, 118.6, 112.3, 103.6, 64.3, 62.5, 60.8, 56.2, 52.0, 48.7, 34.1. HRMS (ESI) m/z Calcd for $C_{31}H_{36}N_4O_7SNa$ $[M+Na]^+$ 631.7051, found 631.7061.

*4.1.6.3. Methyl 3-methoxy-4-(3-(5-(4-(3-nitrobenzyl)piperazin-1-yl) pyridin-2-yl)-4-oxothiazolidin-2-yl)benzoate (**9c**).* Yield: 70%; Pale yellow gummy solid; IR γmax (nujol) 3037, 1739, 1652, 1593, 1541, 1452, 1389, 1378, 1363, 1245, 1165, 1065 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 8.01–8.00 (d, J = 2.8 Hz, 1H, ArH), 7.98–7.96 (d, J = 9.2 Hz, 1H, ArH), 7.88–7.86 (m, 1H, ArH), 7.84 (s, 1H, ArH), 7.74–7.71 (m, 1H, ArH), 7.62–7.60 (d, J = 8.2 Hz, 1H, ArH), 7.57–7.56 (d, J = 2.4 Hz, 1H, ArH), 7.45–7.42 (dd, J = 2.4 Hz, 1H, ArH), 7.37–7.34 (dd, J = 3.2 Hz, 1H, ArH), 7.00–6.98 (d, J = 8.8 Hz, 1H, ArH), 6.72 (s, 1H, thiazolidinone-CH), 4.04–4.01 (dd, J = 1.4 Hz, 1H, thiazolidinone-$CH_2(H_A)$), 3.84–3.81 (m, 1H, thiazolidinone-$CH_2(H_B)$), 3.74 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 3.63 (s, 2H, benzyl-CH_2), 3.36–3.33 (m, 4H, piperazine-CH_2), 3.01–2.99 (m, 4H, piperazine-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.9, 166.2, 158.6, 147.9, 144.6, 142.6, 136.8, 136.0, 135.1, 134.3, 132.5, 131.7, 130.2, 129.3, 125.2, 122.4, 120.1, 118.8, 112.0, 63.9, 62.6, 56.2, 52.1, 48.7, 34.1. HRMS (ESI) m/z Calcd for $C_{28}H_{29}N_5O_6SNa$ $[M+Na]^+$ 586.6248, found 586.6259.

*4.1.6.4. Methyl 4-(3-(5-(4-(3,4-dichlorobenzyl)piperazin-1-yl)pyridin-2-yl)-4-oxothiazolidin-2-yl)-3-methoxybenzoate (**9d**).* Yield: 73%; Pale brown gummy solid; IR γmax (nujol) 3048, 1740, 1658, 1588, 1451, 1392, 1369, 1252, 1178, 1060, 786 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 8.00–7.99 (d, J = 2.6 Hz, 1H, ArH), 7.97–7.95 (d, J = 8.8 Hz, 1H, ArH), 7.74–7.72 (d, J = 8.0 Hz, 1H, ArH), 7.58–7.57 (d, J = 2.2 Hz, 1H, ArH), 7.43–7.41 (dd, J = 2.4 Hz, 1H, ArH), 7.36–7.33 (dd, J = 3.2 Hz, 1H, ArH), 7.31 (s, 1H, ArH), 7.17–7.15 (d, J = 8.0 Hz, 1H, ArH), 6.99–6.97 (d, J = 8.8 Hz, 1H, ArH), 6.73 (s, 1H, thiazolidinone-CH), 4.05–4.02 (dd, J = 1.2 Hz, 1H, thiazolidinone-$CH_2(H_A)$), 3.83–3.80 (m, 1H, thiazolidinone-$CH_2(H_B)$), 3.75 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.64 (s, 2H, benzyl-CH_2), 3.38–3.35 (m, 4H, piperazine-CH_2), 3.03–3.00 (m, 4H, piperazine-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.9, 166.2, 158.7, 144.9, 142.7, 136.1, 135.2, 134.4, 132.5, 131.8, 131.6, 130.1, 129.8, 129.0, 128.6, 125.2, 120.2, 118.6, 112.3, 63.7, 62.6, 56.1, 51.8, 48.8, 34.3. HRMS (ESI) m/z Calcd for $C_{28}H_{28}Cl_2N_4O_4SNa$ $[M+Na]^+$ 610.5173, found 610.5182.

4.2. Biology

The human chronic myelogenous leukemia (CML) cell line, K562, human T cell lymphoblast-like cell line Jurkat and B cell leukemia cell line Nalm6 were selected for the purpose of preliminary anticancer screening of newly synthesized compounds. To assess the cytotoxicity, trypan blue dye exclusion assays, MTT assay were employed as described earlier [22]. Further, cell cycle analysis, mitochondrial membrane potential assay were also performed in order to understand the mode of cell death after treatment with compound **5** [23,24].

4.2.1. Cell lines and culture

Human cell lines, K562, Jurkat (T-cell leukemia) were purchased from National Center for Cell Science, Pune, India. Cells were grown

in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL of Penicillin, and 100 μg of streptomycin/mL and incubated at 37 °C in a humidified atmosphere containing 5% CO_2.

4.2.2. Trypan blue dye exclusion assay

The effect of **5**, **8a**–**f** and **9a**–**d** on cell viability of Nalm6 cells was determined by Trypan blue dye exclusion assay [25]. Nalm6 cells were seeded at a density of 1×10^5 cells/ml, cultured for 24 h and synthesized compounds were added at a concentration of 5, 10 and 20 μM. DMSO (Sigma Aldrich, USA) treated cells were used as vehicle control. Cells were harvested after 48 h and 72 h time intervals and suspended in 0.4% Trypan blue (Sigma Aldrich, USA) and the viable cells were counted using haemocytometer. Experiments were repeated at least 3 times and the values obtained were plotted against different time points (Fig. 2A, C, E).

4.2.3. MTT assay

Cell proliferation was further assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay which is based on the ability of viable cells to metabolize a yellow tetrazolium salt to violet formazan. Exponentially growing Jurkat, K562 and Nalm6 cells (5×10^4 cells/well) were plated 24 well plate and incubated with 5, 10 and 20 μM of compounds **5**, **8a**–**f** and **9a**–**d**, cells were harvested after 48 h and 72 h of treatment and incubated with MTT (0.5 mg/mL) at 37 °C in 96 well plate. The blue MTT formazan precipitate was then solubilized in detergent (50% final concentration of *N*, *N*-dimethylformamide and 10% of sodium dodecyl sulfate). Absorbance was measured at 570 nm using ELISA plate reader. The mean absorbance of culture medium was used as the blank and was subtracted. All measurements were performed in triplicate and each experiment was repeated at least three times.

4.2.4. Cell cycle analysis using fluorescent activated cell sorter (FACS) analysis

Cellular DNA content of Nalm6 cells were treated with different concentration of **5** treated with 5, 10 and 20 μM (Approximately 0.75×10^5 cells/ml), cells were harvested after 48 h, washed with PBS, RNase (50 μg/ml) (Sigma Aldrich, USA) treatment was given and finally stained with propidium iodide [26] (Sigma Aldrich, USA) and subjected to flow cytometry (FACS Verse, BD Biosciences, USA) using Cell Quest Pro Software, excitation 488 nm laser and emission at 560/670 nm. A minimum of 10,000 cells were recorded per sample and histograms were analyzed with Flowing Software (Version 2.5).

4.2.5. Mitochondrial membrane potential assay

Changes of mitochondrial trans membrane potential ($\Delta\psi m$) were analyzed by flow cytometry using a sensitive dye called JC-1(5,5′,6,6′-Tetrachloro-1,1′,3,3′-tetraethylbenzimidazolo carbocyanine iodide (Calbiochem, USA) [23]. Nalm6 cells were treated with compound **5** (5, 10 and 20 μM), cells were harvested after 48 h and incubated with JC-1 (0.5 μM) at 37 °C for 30 min. Finally, cells were washed with 1× PBS and the cells were subjected to flowcytometric analysis, using Cell Quest Pro software an excitation at 488 nm laser and emission at 530/630, 580/610 nm. 10,000 cells were acquired per sample and 2,4-Dinitrophenol (2, 4-DNP) used as positive control. Results were analyzed in WinMDI 2.9 software and data were presented.

4.2.6. Statistical analysis

Statistical analysis was done by using Graph Pad software prism 5.1, values were expressed as mean ± SEM for samples and statistical analysis was performed. One-way ANOVA followed by Dunnett test, in each case experimental samples were compared with control and significance was determined. The values were considered as statistically significant, if the *p*-value was equal to or less than 0.05.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.05.009.

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