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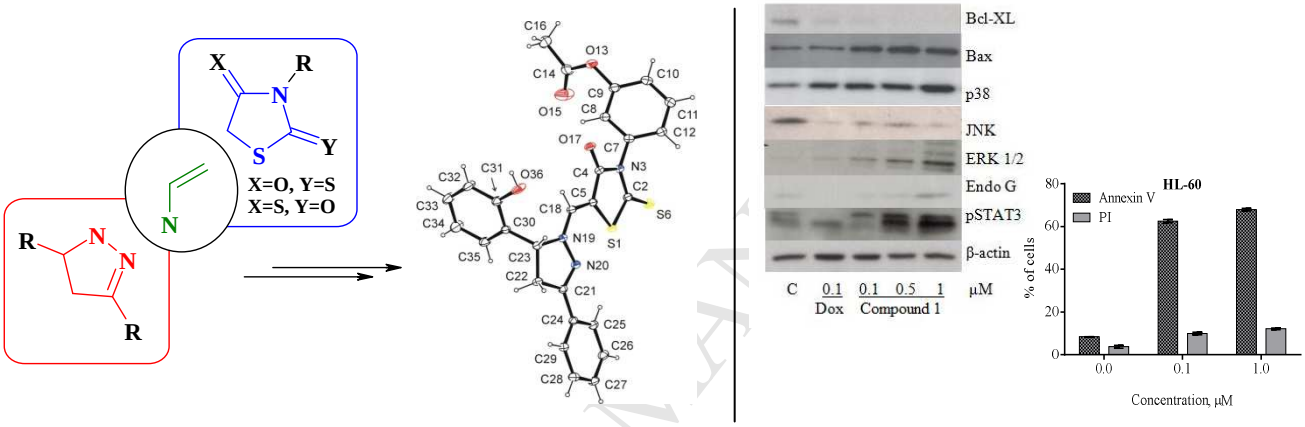

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Abstract

The article presented the synthesis of 5-ene-4-thiazolidinone derivatives with pyrazole core linked by enamine group. The structure and purity of compounds were confirmed by analytical and spectral data including X-ray analysis. Target compounds were screened for their anticancer activity and selective antileukemic action was confirmed. 5-[5-(2-Hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one (compound **1**) was selected as most active agent against HL-60 and HL-60/ADR cell lines; IC_{50}=118 nM / HL-60 with low toxicity towards pseudonormal cells. The mitochondria-depended apoptosis was identified as the main mode of **1** action. Moreover compound's effect induces G_0/G_1 arrest of the treated cells and causes inhibition of cell division and is related with activation of ROS production.

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1. Introduction

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4-Thiazolidinones and related heterocycles are intensively explored for design of new drug-like molecules [1-3]. Despite the variety of 4-thiazolidinone bearing compounds, the search for new antibacterial, antiviral, anti-inflammatory, and antidiabetic agents [4,5] is the main direction associated with the thiazolidinone framework. Nowadays research area is mainly focused on a design of new anticancer agents [6]. A majority of the biologically active 4-thiazolidinones belong to the 5-ylidene (5-ene) derivatives containing the exocyclic double bond. This is reflected in thesis about a crucial role of the C5 substituent (namely 5-ene) in the pharmacological effect [7-11]. Conjugation of the 5-ene fragment to the carbonyl group at C4 position of the thiazolidine core makes the compounds to be electrophilic and potentially reactive due to a possible Michael addition of the nucleophilic protein residues to the exocyclic double bond [12]. This property can characterize 5-ene-4-thiazolidinones as frequent hitters or pan assay interference compounds that are treated as useless in the drug discovery process because of their possible insufficient selectivity [3,13,14]. This view is disproved in many studies and a large number of lead-compounds belong to the mentioned 5-ene-4-thiazolidinones. From another hand, such Michael acceptors are among the most effective activators of the Nrf2 through the Keap1 modification that opens new perspectives in treatment of inflammation and cancer [15,16]. Positive perspective of above mentioned thiazolidinone sub-type is linked to a polypharmacological approach in drug discovery, where the affinity toward various targets is regarded as anadvantage [16]. Moreover, 5-ene-4-thiazolidinones are the examples of privileged scaffolds that can be treated as drug-like molecular structures providing baseline affinity for the whole protein family [17]. Besides, better results in drug design can be expected when responses are evoked by the multipoint interventions in more than one mechanism and in different targets, following a concept of multi-target drugs. The exploitation of 5-ylidene diversity allows achieving such desired combinations, for instance within the hybrid pharmacophore approach [17]. A significant biological potency of compounds bearing 5-ene-4-thiazolidinone fragment in combination with the heterocyclic moieties (such as thiazole, pyrazole, flavone, chromone, furan, etc.) or other molecular fragments was discovered [7,9,19-22]. Such approach can be treated as a perspective tool for improving selectivity.

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Numerous papers and patents are devoted to design and development of new anticancer agents based on 5-ene-4-thiazolidinones [1,6]. Research in this field can be outlined in the following directions: *i)* screening of the libraries of compounds for further hits optimization; *ii)* design of high affinity ligands to "validated" anticancer targets; *iii)* the creation of the hybrid molecules combining several attractive scaffolds [23]; *iv*) search for anticancer agents among compounds/drugs with other biological activities (anti-inflammatory, antidiabetic, antimicrobial, etc.) [4,5,24].

Despite a long list of potential and validated bio-targets for new anticancer agents, there is no systematization of the 4-thiazolidinones as anticancer agents. It was discovered that 5-ene-4-thiazolidinones can act as: 1) inhibitors of the antiapoptic protein-protein interactions – between Bcl-2 and Bax family, as well as inhibitors of their interaction with the receptors' domains [25,26]; 2) inhibitors of JSP-1 – "atypical" dual-specific phosphatases family member (JNK-stimulating phosphatase-1) [26]; 3) COX inhibitors [28] because of their potential anticancer activity; 4) inhibitors of insulin-like growth factor-1 receptor [29]; 5) selective inhibitors of extracellular signal-regulated kinases-1 and 2 (ERK1/2) [30]; 6) modulators of estrogen-related receptor-α (ERR-α) [31]; 7) Pim-1 and Pim-2 inhibitors [32]; 8) inhibitors of CDK1 [33,34] and CDK1/cyclin B [35]; 9) inhibitors of Polo-like kinase 1 (Plk1) [36]; 10) agents with SHP-2 inhibitory action (SHP-2, a non-receptor protein tyrosine phosphatase that mediates cell signaling via growth factors and cytokines acting via the RAS/MAP kinase pathway [37]; 11) tumor necrosis factor (TNFα) inhibitors [38]; 12) integrin $\alpha_v\beta_3$ receptor inhibitors [39] etc. Besides, the 5-ylidene rhodanines can act as "Myc-Max compounds" that inhibit or reverse association between c-Myc and Max [40], etc.

Many chemotherapeutics exert the anticancer activity by triggering apoptotic cell death and exhibit excellent antiproliferative properties [41]. Despite high affinity to different molecular targets, apoptosis induced by 4-thiazolidinones has been demonstrated in various cancer cells [42,43]. The apoptosis-depended mode of action is mainly related here with the inhibition of Bcl-2/Bcl-X_L function, although other effects of 5-ene-4-thiazolidinones in the apoptotic signaling pathway involving mitochondria-mediated apoptosis have been described [44]. 4-Thiazolidinones also decrease mitochondrial membrane potential in leukemic cells that is one of the most important mechanisms for the mitochondrial mediated apoptotic cell death [45]. As an example, MKT 077 (1-ethyl-2-[[3-ethyl-5-

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(3-methyl-2(3H)-benzothiazolylidene)-4-oxo-2-thiazolidinylidene]methyl]pyridinium chloride) has been reported as an antitumor agent with apoptosis related mechanism of action which is related to its differential interaction with the Hsp70 (Heat shock protein 70 kDa) allosteric states and with the reactivation of p53 function [46].

Evolution of the adaptive mechanisms provides cancer cells with the ability to evade apoptotic execution and bestows a survival advantage upon them. Most tumors develop a multi-drug resistance that is often associated with the over-expression of the membrane permeability-glycoprotein (P-gp). It is firmly established that P-gp-mediated multi-drugresistance is a major obstacle to a successful cancer chemotherapy [47]. Data were obtained suggesting that 4-thiazolidinones (e.g. MMPT (5-[(4-methylphenyl)methylene]-2-(phenylamino)-4(5*H*)-thiazolone) and DBPT (5-(2,4-dihydroxybenzylidene)-2-(phenylimino)-1,3-thiazolidine) inhibit growth of drug-resistant cancer cells (human non-small-cell lung and colon cancer) independent on P-gp and p53 status and induce apoptosis [48].

Cancer cells are characterized by a loss of differentiation control and changes in cell cycling. The cell cycle is important in cancer treatment because many therapies work the best when cells are actively or rapidly dividing. For example, some chemotherapy drugs work by targeting cells in a particular phase of the cell cycle, such as G_1, S or G_2 [49]. Anticancer effect of 5-ene-4-thiazolidinones can be achieved by a reversible blocking of cell cycle progression at the G_2/M phase border that leads to induction of apoptosis [33]. FACS analysis of the action of 5-arylfurfurylidene-4-thiazolidinone-3-carboxylic acids possessing strong antiproliferative activity towards human leukemia cells showed a remarkable accumulation of sub-ploid cells, the sub-G_1 phase (G_0/G_1) followed by a decline of both G_1 and G_2/M phases, and the observed growth inhibition could be due to apoptosis induction [34]. The related 5-ene-4-thiazolidinones with heterocyclic fragments induced cell cycle arrest of leukemic cells at the G_2/M phase and increased number of cells in the sub-G_1 phase leading to cell death, as a result of depolarization of the mitochondrial membrane potential. Similar data were obtained at the action of 2-heterylamino-4-thiazolidinones that induced dissipation of the mitochondrial membrane potential, as well as redox changes in treated HT29 cells accumulated in the G_2/M and sub-G_0/G_1 phases of cell cycle [11]. Treatment of cancer cells with MMPT and DBPT also led to a time-dependent arrest of cells in the

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G_2/M phase. 5-Ene-4-thiazolidinones may act as an ATP-competitive inhibitors of Plk1 (a key regulator of mitotic progression and cell division in the eukaryotes) in a concert with cyclin-dependent kinase 1–cyclin B1 and Aurora kinases to orchestrate a wide range of critical cell cycle events leading to a prometaphase-like mitotic (G_2/M) arrest [36,50].

The antileukemic effect of variety 5-ene-4-thiazolidinones is one of the most prominent directions that was demonstrated in our studies [2,7,9,21], as well as in investigations of other scientists [34]. The crucial role of the C5-ene fragment of 4-thiazolidinones in the antileukemic effect was also confirmed, and its complication was shown as a potent direction for compounds optimization.

Present work is an extension of our ongoing efforts towards a search for new 4-thiazolidinone-based anticancer agents. Here we addressed the synthesis of new 5-ene-4-thiazolidinones, evaluation of their anticancer effects in the leukemia and carcinoma cells *in vitro,* and study of signaling pathways involved in apoptosis induction in tumor cells treated with the synthesized 4-thiazolidinones.

2. Results

2.1. Chemistry

Design of molecular structure of target compounds was based on our former findings [9,10,51], as well as on following items: *i*) belonging of target compounds to the 5-ene-4-thiazolidinone sub-type; *ii*) combination of pyrazoline and thiazolidinone cores within hybride pharmacophore approach; *iii*) introduction of substituents in N3 position of main core – as a phase of compounds modification aimed at a decrease of its general toxicity [52]; *iv*) geometry of molecular structures (C5 ring, thiazolidinone core and N3 residue) meets the suggesting that the three-ring system might be required for the anticancer activity [48,53]; *v*) introduction of OH group in ortho position of the phenyl moiety (C5 fragment) is desirable for anticancer effect realization [54].

Synthesis of the 4-thiazolidinone derivatives was performed according to earlier described 2-stages protocol based on the modification of methylene group of the thiazolidinone core [55,56]. The procedure included condensation with triethyl orthoformate, and the obtained 5-ethoxy-4-thiazolidinones were converted into the appropriate enamines under the reaction with appropriate 3,5-

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diaryl-2-pyrazolines [51] (Fig. 1). Rhodanine and isorhodanine were used as basic cores in order to establish structure-activity relationships.

Fig. 1.

Structure of the synthesized compounds was confirmed by the elemental analysis and spectroscopic data (^{1}H NMR, ^{13}C NMR and LCMS). ^{1}H NMR spectra of synthesized compounds showed characteristic patterns of an AMX system for protons at positions 4 and 5 of pyrazoline ring. The proton of the methylene group (=CH) showed a singlet at 7.19-7.95 ppm (*Z*-isomer), and broad singlet of the NH proton of the thiazolidinone ring appeared at 12.32-12.97 ppm.

The X-ray analysis (Fig. 2) revealed that the crystal of compound **1** is a channel-forming solvate crystal with strongly disordered ethanol molecules. There are four symmetry-independent molecules A – D and three and a half solvent molecules in the asymmetric part of the unit cell. The solute molecules differ strongly in terms of conformation and the differences are related to the arrangement of two cyclic systems, *i.e.* 2-hydroxyphenyl and 3-acetoxyphenyl (Fig. 3). The other systems in 5-(3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-2-thioxothiazolidin-4-one moiety, *i.e.* 2-thioxothiazolidin-4-one, 5-dihydropyrazole and phenyl group, in molecules A – D are approximately coplanar. 2-Hydroxyphenyl and 3-acetoxyphenyl rings form with the mean plane of 5-(3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-2-thioxothiazolidin-4-one the dihedral angles of 88.65(6), 86.11(7), 71.98(5) i 70.20(6)° and 86.80(6), 52.35(6), 103.37(7), 113.45(7)°, respectively.

Fig. 2.

The arrangement of the mentioned systems are also described with torsion angles C2–N3–C7–C8 and N19–C23–C30–C31 which reveal values of -95.1(3), 123.7(3), 76.3(3), -114.4(3)° and -84.4(3), -98.3(3), -87.3(3), -90.7(2)°, respectively.

In molecules A and C the carbonyl oxygen atom O15 of acetoxyl group is disordered and takes two alternative positions *a* and *b* (Fig. 3) with site occupancy factors in molecule A of 0.910(7) and 0.090(7), in molecule C of 0.514(9) and 0.486(9), respectively. In molecule D the whole acetoxyl group is disordered where the atoms O13D, C14D, O15D, C16D (Fig. 2 and 3) take two alternative positions with site occupancy factors of 0.911(3) and 0.089(3), respectively.

Fig. 3.

2.2. Screening of anticancer activity

In vitro screening of anticancer activity of the synthesized compounds towards human promyelocytic leukemia cells of HL-60 line and human ovarian carcinoma cells of Skov3 line was performed by the MTT assay. Doxorubicin was used as a reference drug in positive control. Tested compounds were added to cultured cells in different concentrations (0 – 10.0 μM) and the cells were treated for 72 h. The screening results were expessed as IC_{50} and outlined in the Table 1. The tested compounds possessed different antiproliferative action that was generally weaker comparing with the doxorubicin, although some of them possess micromolar meaning of the IC_{50}.

Table 1

N-substituted derivatives were characterized by higher level of activity (comparison of compounds **8** and **9**), presence of the aromatic moiety is better in comparison with the aliphatic one (compounds **7**, **4**, **3** and **1**, **2**). It should be stressed that position of the substituent in the benzene fragment (N3-possition of thiazolidinone core) is of great significance here (e.g. different level of activity of compounds **1** and **2**). These findings need further investigation since there were no such peculiarities when the activities of the compounds **18** and **12**, **13** were compared. It was found that OH group in the ortho position (R^2, Fig. 1) was optimal (comparison of **12**, **13** and **14**), while a complication of arylidene fragments of the pyrazoline ring could be considered as a direction to increase of the anticancer activity (compounds **2**, **11** and **12**). Compounds based on isorhodanine core (**19**, **20**) possessed higher antiproliferative activity that argues a relevance of this direction.

In general, tested compounds were less active towards Skov3 cells growing in a substrate-dependent manner comparing with such activity towards HL-60 cells growing in suspension: compound **18**: IC_{50} = 0.163 μM versus 0.400±0.052 μM, compound **2**: IC_{50} = 0.244 μM versus 9.400±2.04 μM, and compound **3**: IC_{50} = 0.341 μM versus 2.880±0.192 μM, correspondingly.

Among tested compounds, **1** and **8** possessed the highest antiproliferative activity (IC_{50} 0.12 and 0.1 μM, correspondingly) towards human leukemia HL-60 cells, however, they were not affecting substrate-dependent human ovarian carcinoma cells of Scov3 line. The compound **1** was selected for further in depth study, and it was screened in different concentrations (0.01-10.0 μM) towards a set of

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the mammalian tumor cell lines of different origin. A 72 h continuous drug exposure protocol was used with the MTT assay.

In vitro screening was held towards the pseudonormal cell lines (HEK 293T, KS 483) and L929 transformed cell line, all growing in a substrate-dependent manner. There was no inhibition of cell viability observed in the case of testing HEK 293T and KS 483 cells (Fig. 4). Compound **1** also did not possess toxic action towards L929 cells. Thus, proliferation of the pseudonormal cells was not inhibited under the effect of compound **1**.

Fig. 4.

The anticancer effect *in vitro* of the compound **1** was also studied using human leukemia Jurkat T-cells, murine leukemia L1210 cells, and human breast carcinoma MCF-7 cells. It was found that *(Z)*-5-[5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one possessed a cytotoxic action towards L1210 cells (IC_{50} = 0.7 μM) and Jurkat cells (IC_{50} = 1.1 μM), however, its toxicity towards MCF-7 cells was expressed only in high doses (IC_{50} = 8.3 μM), suggesting leukemia cell specificity of its action.

Following prominent effect of compound **1** on growth of human leukemia HL-60 cells, the HL-60/ADR (adriamycin-resistant cells with over-expression of P-glycoprotein) sub-line of these cells was involved in the study. Compound **1** was less effective toxicant for the HL-60/ADR cells (IC_{50} = 1.00 μM) than for their wild type (IC_{50} = 0.12 μM) (Fig. 4), such effect was much more prominent than the effect of doxorubicin – "golden standart" in the chemotherapy (IC_{50} = 8.8 μM). The mechanisms responsible for leukemia cell specificity of cytotoxic action of the **1** should be studied additionally.

The cytotoxic effect of compound **1** towards HL-60 and HL-60/ADR human leukemia cells was dose- and time-dependent (3, 6, 24, 48 and 72 h). It demonstrated the antiproliferative action towards HL-60 as soon as in 6 h after its addition to cultured cells (IC_{50} = 0.5 μM), while its toxicity towards HL-60/ADR was prominent only after 24 h (IC_{50} = 1.0 μM) (Fig. 5).

Fig. 5.

2.3. Mode of action

The anticancer effect of cisplatin and doxorubicin ("classical" anticancer drugs) is accompanied by an increase in the intracellular level of the reactive oxygen species (ROS) that may contribute to their

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therapeutic effect [57]. To investigate whether the 4-thiazolidinone derivatives kill tumor cells by free radicals/ROS-depended manner, we have monitored the **1** action using well known antioxidants – N-acetylcysteine (NAC) and ascorbic acid (AA). The NAC is an analogue and precursor of the glutathione and it displays cancer preventive properties not only at early stages of carcinogenesis process but also during its advanced stages. NAC is a source of sulfhydryl groups in cells and scavenger for free radicals. It interacts with ROS such as $OH^{\cdot}$ and H_2O_2, thus, preventing apoptosis and promoting cell survival [41]. NAC (1.0 mM) had a protective effect on the cytotoxic action of compound **1** towards leukemia cells over a period of 72 h exposure (Fig. 6).

Fig. 6.

Ascorbic acid deficiency is accompanied by an increased oxidative stress and tissue injury including oxidant-induced necrotic cell death. It prevents oxidative modification of both cytosolic and membrane components of cells [58]. We found that AA (25.0 μM) under compound **1** treatment significantly enhanced survival of HL-60 and HL-60/ADR cells compared to the level of compound **1** action (Fig. 6). Thus, cytotoxic effect of compound **1** in tumor cells was blocked by the NAC and AA which inhibited the action of ROS. The ROS are an effective weapon used by the anticancer chemotherapeutics against tumor cells [11]. Probably, the compound **1** induced the accumulation of ROS in human leukemia HL-60 and HL-60/ADR cells that causes its antitumor effect under most probably mitochondria-mediated apoptosis.

In the next experiments, the apoptosis-inducing potential of compound **1** was studied in more detail. Supravital double staining with FITC-conjugated Annexin V and propidium iodide of the HL-60 cells treated for 48 h with **1** (0.1 and 1.0 μM) showed that tested compound induced apoptosis in these leukemia cells (Fig. 7B).

Western-blot analysis of proteins of treated cells was used to explore the molecular mechanisms underlying the pro-apoptotic activity of the compound **1**. The lysates were obtained from human promyelocytic leukemia HL-60 cells treated for 48 h with *(Z)*-5-[5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one (**1**) (0.1; 0.5; 1.0 μM) or doxorubicin (0.1 μM), used as a positive control. It was suggested that antitumor action of the 4-thiazolidinones is associated with their affinity to pro- and anti-apoptotic members of the family of Bcl-

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2 proteins [25]. Here we have found that under the action of **1** the level of the pro-apoptotic protein Bax was increased, while the level of the anti-apoptotic protein Bcl-XL was decreased (Fig. 7A).

Thus, we have studied activation of the MAPKs such as JNK, ERK and p38 in HL-60 cells treated with the compound **1** (Fig. 7A). It was found that compound **1** markedly stimulated phosphorylation of the MAPK p38 at 48 h after the start of treatment. We have found that compound **1** inhibited c-Jun N-terminal kinase (JNK) phosphorylation that can mediate inhibition of c-Jun phosphorylation [48]. Inhibition of JNK is accompanied by the activation of STAT 3 [52], we have found the increasing of pSTAT3 expression (Fig. 7A). Besides, we have found the activation of ERK1/2 and Endo Gunder the action (48 h) of compound **1**.

Fig. 7.

A loss of proper control of the cell cycle in the mammalian cells is one of the main engines leading to cellular transformation [59]. We studied the effect of compound **1** on cell cycling in HL-60 leukemia cells treated with that compound for 8 and 48 h. The obtained flow cytometry data (Fig. 8A) demonstrate that compound **1** affects HL-60 cell cycle and increases a percentage of cells in the G_0/G_1 phase, while the doxorubicin increased the number of the G_2/M phase cells [60]. Thus, compound **1** cause the G_0/G_1arrest of cells that differs it from the pharmacological profile of the related 4-thiazolidinones.

As shown in Fig. 8B, the treatment of HL-60 leukemia cells with compound **1** caused an increase in the level of p21 and Cdk1 proreins. p21 directly inactivates Cdk2 by blocking cell transition from the G_1 to S phase of cell cycle. That was proved by the flow cytometry results (Fig. 8A).

Fig. 8.

We found that *(Z)*-5-[5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one (**1**) inhibited phosphorylation of the Rb (Retinoblastoma) protein and decreased the amount of the Cyclin D3 in HL-60 leakemia cells (Fig. 8B). The activity of this protein is required for the G_1/S transition in the cell cycle. This correlates with the obtained results of the Western-blot analysis of expression of pRb (Fig. 8B).

Summarizing, compound **1** induces apoptosis with activation of Bax, MAPKs, Endo G by mitochondria-mediated pathway and causes G_0/G_1 arrest in cell cycle of the treated cells.

3. Discussion

In vitro screening of the anticancer activity of synthesized 4-thiazolidinone derivatives towards human promyelocytic leukemia cells of HL-60 line showed that among tested compounds *(Z)*-5-[5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one **1** and *(Z)-5*-[5-(4-dimethylaminophenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one **8** possessed the highest antiproliferative activity (IC_{50} 0.12 and 0.10 μM, correspondingly), however, they were not affecting human ovarian carcinoma cells of Scov3 line growing in a substrate-dependent manner. Tested compounds based on the isorhodanine core possessed higher antiproliferative activity that argues the relevance of this direction. Moreover, modification of pyrazoline core could be considered as a perspective direction for increase of the anticancer activity of new N-substituted 5-ene-thiazolidinones. Proliferation of the pseudonormal cells was not inhibited under the effect of compound **1**. Similar results were obtained when the rat myoblast-derived cells of L-6 line were treated [51]. Recently, we described a marked anti-trypanosomal activity of such compounds [50]. Taken together, these data may suggest an existence of common mechanisms of their cytotoxic activity towards tumor and protozoa cells [5,24]. Thus, the synthesized substances can be considered as so-called multi-target compounds (see above).

A resistance to the anthracycline antibiotics and other chemotherapeutics due to P-glycoprotein (P-gp)-mediated export of xenobiotics is a frequent problem in cancer treatment. Compound **1** was more effective toxicant for the HL-60/ADR cells (IC_{50} = 1.00 μM) than the effect of doxorubicin (IC_{50} = 8.8 μM). It was shown that structurally related compounds were not inhibiting P-glycoproteins and acted independently on the P-glycoprotein mechanisms [47,48,53]. Such characteristics could be inherent to the compounds under this study, since their structure does not correspond to structural requirements of inhibitors of P-glycoproteins [53]. Presented findings confirm our statement about leukemia-specific targeting of the 5-ene-4-thiazolidinone derivatives [7,9].

The mechanisms responsible for cytotoxic action of the compound **1** were also studied. The anticancer effect of cisplatin and doxorubicin is known to be accompanied by an increase in the intracellular level of the reactive oxygen species that contribute to their therapeutic effect [57]. High

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levels of ROS in mitochondria (as a primary source of ROS) can result in free radical attack of membrane phospholipids and cause mitochondrial membrane depolarization [61]. Moreover, ROS play a key role in the mitochondria-mediated apoptosis. Thus, compound **1** induced the accumulation of ROS in human leukemia HL-60 and HL-60/ADR cells that causes its antitumor effect under most probably mitochondria-mediated apoptosis. This is an irreversible step associated with the attack of membrane phospholipids, release of mitochondrial factors, and triggering caspase cascades [62]. It was reported that ROS level increased under the action of 5-ene-4-thiazolidinones in HT29 and CEM cells, and it was inhibited by the AA [11,63]. Besides, it was shown that 5-(4-hydroxy-3-methoxybenzylidene)-2-thioxo-4-thiazolidinone significantly elevated the Parkin and Miro2 expression levels in the acute MPTP-treated mice, and stabilized mitochondrial membrane potential and ATP synthesis in MPP+-treated Neuro-2a cells [64]. It is also worth to note about a search for potent antioxidants among the 4-thiazolidinones including compounds with the anticancer potential [65]. 2-Benzo[*d*]isothiazolylimino-5-benzylidene-4-thiazolidinones were shown to possess high antioxidant effect, NO lowering and GAGs restoring capability, and also reduced the production of the metalloproteinases and NF-κB expression [66]. It is desired that new anticancer agents do not only induce the apoptosis of tumor cells leading to quick phagocytosis of the remnants of tumor cells by the macrophages, but also possess a capability to diminish the negative side effects of applied chemotherapy. Thus, novel 5-ylidene-4-thiazolidinone derivatives could be of great use in that regard.

It was reported that the anticancer effects of related 4-thiazolidinones could be realized by apoptosis induction in different ways – through PARP-, MAPK-, JNK-, Bcl-2-, CDK1/cyclin B inhibition or be dependent on caspase cascades [6,48].

It was suggested that antitumor action of 4-thiazolidinones is associated with their affinity to pro- and anti-apoptotic members of the family of Bcl-2 proteins [25]. These proteins can stimulate the mitochondrial outer membrane permeabilization and a release of proteins of the inter-membrane space into the cytosol. After induction of the apoptotic stimuli by the anticancer drugs, the pro-apoptotic proteins of that family, including Bax, Bak and Bad, are induced in accordance with their sensitivity to the anticancer drugs [67]. The homodimerized Bax acts on the VDAC (Voltage-dependent anion channel) localized in the outer membrane of mitochondria, thus, resulting in a release of cytochrome *c*

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which activates the caspase cascade [68,69]. It was also reported that a release of cytochrome *c* is inhibited by the antiapoptotic proteins, such as Bcl-2 and Bcl-X_L [68]. Here we have found that under the action of compound **1** the level of the pro-apoptotic Bax protein was increased, while the level of the antiapoptotic Bcl-XL protein was decreased.

It is known that cellular stresses and stimuli can induce apoptosis via modulating the mitogen-activated protein kinases (MAPK) signaling pathway [48,70] (inhibition of JNK, and activation of p38, ERK under the action of **1**). The activated p38 was shown to phosphorylate p53 [46]. Inhibition of JNK is accompanied by the activation of STAT 3 (pSTAT3 expression increasing). The activation/inhibition of JNK modulates the phosphorylation of mitochondrial proteins during apoptosis. ERK can regulate cell growth at the transcription level of an early response gene, such as c-*fos*, mediated by STAT3 (Ser 727) activation. Activation of ERK also causes phosphorylation of the nuclear substrate STAT3 that correlates well with our results. Activation of the ERK1/2 and EndoG leads to a release of cytochrome *c* and subsequent formation of the apoptosomes through the mitochondria-mediated apoptosis pathway [70]. It was also reported that 4-thiazolidinone derivatives (e.g. MMPT) induced apoptosis in lung cancer cells by caspase-3, -8, -9, and poly(ADP-ribose) polymerase cleavage and cytochrome *c* release from the mitochondria [48]. Besides, a loss of proper control of cell cycle in the mammalian cells is one of the main engines leading to cellular transformation [59]. Compound **1** causes the G_0/G_1arrest of cells that differs it from the pharmacological profile of the related 4-thiazolidinones.

Treatment of the HL-60 leukemia cells with compound **1** caused an increase in the level of p21 and Cdk1 proteins. As a proliferation inhibitor, p21 can play an important role in preventing tumor development. The mechanisms by which p21 may promote apoptosis could be related to its ability to affect the components of the DNA repair machinery [59]. P21 directly inactivates Cdk2 by blocking the transition from the G_1 to S phase of cell cycle. Our findings demonstrate a correlation between the p21 expression and induction of the pro-apoptotic Bax protein. P21 induces the expression of the pro-apoptotic Bax protein to the levels sufficient to inhibit the apoptotic Bcl-2 inhibitor that represents its counterpart [71]. Elevation in the cyclin-dependent kinase 1 (Cdk1) plays a key role in control of the eukaryotic cell cycle by modulating centrosome cycle, as well as a mitotic onset. It promotes the G_2/M

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transition and regulates G_1 progress and the G_1-S transition via association with the multiple interphase cyclins [71,72].

We have also found inhibition of phosphorylation of Rb and decrease in the amount of Cyclin D3 in the HL-60 leukemia cells under the action of compound **1**. During cell cycle, phosphorylation of the Rb protein along the G_1 phase is exerted by the CDK2/CyclinE complex and leads to a release of the E2F transcription factor that activates expression of genes needed for the S phase. Rb is a tumor suppressor protein that in the dephosphorylated form does not release E2F, thus, avoiding cell cycle progression by inhibiting the E2F activity [72]. The inhibition of Cyclin D3 correlates with the G_0/G_1 arrest in these cells. Cyclin D3 was shown to interact with the cyclin dependent kinases 4 and 6 (CDK4/6) and was involved in phosphorylation of the tumor suppressor Rb protein [73]. In conclusion, the compound **1** induces apoptosis by the mitochondria-mediated pathway and causes G_0/G_1arrest in cell cycle of the leukemia cells.

4. Conclusion

The panel of 5-ene-4-thiazolidinones was synthesized and screened for their anticancer activity *in vitro*. (*Z*)-5-[5-(2-Hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one (**1**) was selected as most active agent with a selective action against the leukemic cells. It inhibits growth of human leukemia cells (HL-60 line) being applied even in the nanomolar concentrations (IC_{50} = 118 nM). In addition, it possesses low toxicity towards the pseudonormal cells. The obtained data suggest the mitochondria-depended apoptosis as the main mode of **1**'s action; compound **1** also induces the G_0/G_1arrest of the treated cells and causes inhibition of cell division.

4. Experimental section

4.1. Chemistry

4.1.1. Materials and methods

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2-Thioxo-4-thiazolidinone (rhodanine) and N3-substituted-rhodanines, 4-thioxo-2-thiazolidinone (isorhodanine) [74], 3,5-diaryl-4,5-dihydro-1*H*-pyrazole [75] were used as starting reagents and prepared according to the method described previously.

Melting points of newly synthesized compounds were measured in open capillary tubes on a BUCHI B-545 melting point apparatus and are uncorrected. The elemental analysis (C, H, N) were performed using a Perkin Elmer 2400 CHN analyzer. Analyses indicated by symbols of the elements or functions were within ± 0.4% of the theoretical values. The ^{1}H NMR spectra were recorded on Varian Gemini 400 MHz and ^{13}C NMR spectra on Varian Mercury-400 100 MHz in DMSO-d_6 using tetramethylsilane as an internal standard. Chemical shifts are reported in ppm units with use of δ scale. Mass spectra were obtained using electrospray ionization techniques on an Agilent 1100 Series LCMS. Analytical HPLC were performed on an Agilent 1100 HPLC with Diode Array Detection. Purity of all compounds was determined to be ≥ 95% by the HPLC. The peak purity was checked with the UV spectra.

4.1.2. General procedure of synthesis of 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-2-thioxothiazolidin-4-ones and 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-4-thioxothiazolidin-2-ones.

A mixture of 5-ethoxymethylene-2-thioxothiazolidin-4-one or 5-ethoxymethylene-4-thioxothiazolidin-2-one (5 mmol) [51] and appropriate 3,5-diaryl-4,5-dihydro-1*H*-pyrazole (5 mmol) was refluxed in 10 mL of ethanol during 1 h. After cooling, the crystalline product was separated by filtration, washed with ethanol, and dried. Re-crystallization from DMF:EtOH (1:1) rendered the target product in a pure form.

4.1.2.1. (Z)-5-[5-(2-Hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one (**1**). Spectral and analytical data are described [51].

4.1.2.2. (Z)-5-[5-(2-Hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-(4-acetoxyphenyl)-2-thioxothiazolidin-4-one (**2**). Yield 74%, mp 277-279 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.03 (s,

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1H, OH), 7.90 (d, 2H, *J* = 7.2 Hz, arom), 7.56-7.62 (m, 3H, arom), 7.46 (s, 1H, =CH), 7.32 (d, 2H, *J* = 8.5 Hz, arom), 7.21-7.28 (m, 4H, arom), 6.84-6.92 (m, 2H, arom), 5.76 (dd, 1H, *J* = 11.5, 6.3 Hz, C$\underline{H_2}$CH), 3.91 (dd, 1H, *J* = 18.0, 11.7 Hz, C$\underline{H_2}$CH), 3.51 (dd, 1H, *J* = 18.2, 6.4 Hz, $CH_2C\underline{H}$), 2.29 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.9, 169.0, 165.9, 159.9, 155.2, 150.3, 133.3, 133.2, 131.1, 130.1, 130.0, 129.9, 129.2, 129.1, 127.1, 124.9, 122.4, 119.2, 115.9, 93.1, 62.0, 40.8, 20.9. LCMS (ESI+) *m/z* 516 $(M+H)^+$. Calcd. for $C_{27}H_{21}N_3O_4S_2$: C, 62.90; H, 4.11; N, 8.15; Found: C, 62.70; H, 4.40; N, 8.00%.

4.1.2.3. (Z)-5-[5-(2-Hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-methyl-2-thioxothiazolidin-4-one (**3**). Yield 72%, mp 313-315 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.02 (s, 1H, OH), 7.84-7.90 (m, 2H, arom), 7.54-7.60 (m, 3H, arom), 7.43 (s, 1H, =CH), 7.20-7.25 (m, 2H, arom), 6.88 (d, 1H, *J* = 7.7 Hz, arom), 6.86 (t, 1H, *J* = 7.5 Hz, arom), 5.73 (dd, 1H, *J* = 11.6, 6.5 Hz, C$\underline{H_2}$CH), 3.88 (dd, 1H, *J* = 18.1, 11.8 Hz, C$\underline{H_2}$CH), 3.51 (dd, 1H, *J* = 18.1, 6.5 Hz, $CH_2C\underline{H}$), 3.28 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.2, 165.9, 159.7, 155.2, 133.0, 131.0, 130.0, 130.0, 129.1, 129.0, 127.0, 124.9, 119.2, 115.9, 92.9, 62.0, 40.6, 30.6. LCMS (ESI+) *m/z* 396 $(M+H)^+$. Calcd. for $C_{20}H_{17}N_3O_2S_2$: C, 60.74; H, 4.33; N, 10.62; Found: C, 60.90; H, 4.50; N, 10.50%.

4.1.2.4. (Z)-5-[5-(2-Hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-ethyl-2-thioxothiazolidin-4-one (**4**). Yield 78%, mp 234-236 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.99 (s, 1H, OH), 7.86 (m, 2H, Hz, arom), 7.53-7.60 (m, 3H, arom), 7.40 (s, 1H, =CH), 7.23 (d, 2H, *J* = 6.4 Hz, arom), 6.88 (d, 1H, *J* = 6.5 Hz, arom), 6,85 (t, 1H, *J* = 7.1 Hz, arom), 5.72 (dd, 1H, C$\underline{H_2}$CH, *J* = 11.1, 6.2 Hz), 3.96 (m, 2H, C$\underline{H_2}$$CH_3$), 3.90 (dd, 1H, C$\underline{H_2}$CH, *J* = 18.1, 11.5 Hz), 3.50 (dd, 1H, $CH_2C\underline{H}$, *J* = 18.2, 6.2 Hz), 1.09 (t, 3H, *J* = 6.7, Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.5, 166.3, 160.4, 155.8, 133.7, 131.7, 130.6, 129.7, 129.6, 127.7, 125.5, 119.9, 116.5, 93.3, 62.5, 41.1, 31.0, 12.6. LCMS (ESI+) *m/z* 410 $(M+H)^+$. Calcd. for $C_{21}H_{19}N_3O_2S_2$: C, 61.59; H, 4.68; N, 10.26; Found: C, 61.70; H, 4.80; N, 10.10%

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4.1.2.5. (Z)-5-[5-(2-Hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-(pyridin-3-yl)-2-thioxothiazolidin-4-one (**5**).Yield 72%, mp 292-294 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.03 (s, 1H, OH), 8.63 (d, 1H, *J* = 4.7 Hz, arom), 8.50 (brs, 1H, arom), 7.90 (d, 2H, *J* = 6.5 Hz, arom), 7.80 (d, 1H, *J* = 8.2 Hz, arom), 7.55-7.61 (m, 4H, arom), 7.45 (s, 1H, =CH), 7.22-7.26 (m, 2H, arom), 6.89 (d, 1H, *J* = 7.8 Hz, arom), 6.87 (t, 1H, *J* = 7.5 Hz, arom), 5.77 (dd, 1H, *J* = 11.6, 6.7 Hz, C$\underline{H_2}$CH), 3.92 (dd, 1H, *J* = 17.9, 11.6 Hz, C$\underline{H_2}$CH), 3.55 (dd, 1H, *J* = 17.9, 6.2 Hz, CH$_2$C$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.0, 165.7, 160.2, 155.2, 149.5, 149.4, 136.7, 133.4, 132.9, 131.2, 130.1, 130.0, 129.1, 129.0, 127.1, 124.9, 123.9, 119.3, 115.9, 93.0, 62.1, 41.0. LCMS (ESI+) *m/z* 459 $(M+H)^+$. Calcd. for $C_{24}H_{18}N_4O_2S_2$: C, 62.86; H, 3.96; N, 12.22; Found: C, 63.00; H, 4.10; N, 12.30%.

4.1.2.6. (Z)-5-[5-(2-Hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-trifluoromethyl-phenyl)-2-thioxothiazolidin-4-one (**6**). Yield 81%, mp 276-278 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ10.03 (s, 1H, OH), 7.90 (d, 2H, *J* = 5.9 Hz, arom), 7.83 (d, 1H, *J* = 8.0 Hz, arom), 7.72-7.77 (m, 2H, arom), 7.64 (d, 2H, *J* = 8.2 Hz, arom), 7.59 (d, 2H, *J* = 6.7 Hz, arom), 7.46 (s, 1H, =CH), 7.22-7.26 (m, 2H, arom), 6.89 (d, 1H, *J* = 8.3 Hz, arom), 6.86 (t, 1H, *J* = 6.9 Hz, arom), 5.77 (dd, 1H, *J* = 11.4, 6.2 Hz, C$\underline{H_2}$CH), 3.55 (dd, 1H, *J* = 18.1, 11.7 Hz, C$\underline{H_2}$CH), 3.51 (dd, 1H, *J* = 18.0, 5.9 Hz, CH$_2$C$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.8, 165.7, 160.1, 155.2, 136.8, 133.9, 131.2, 130.2, 130.1, 130.0, 129.7 (q, *J* = 32 Hz), 129.1, 129.1, 127.1, 126.0, 125.7, 125.0 (q, *J* = 272 Hz), 124.9, 119.2, 115.9, 93.1, 62.0, 41.1. LCMS (ESI+) *m/z* 526 $(M+H)^+$. Calcd. for $C_{26}H_{18}F_3N_3O_2S_2$: C, 59.42; H, 3.45; N, 8.00; Found: C, 59.60; H, 3.60; N, 7.80%.

4.1.2.7. (Z)-3-(5-[5-(2-Hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-2-thioxothiazolidin-4-one-3-yl)-propionic acid ethyl ester (**7**). Yield 75%, mp 258-260 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.02 (s, 1H, OH), 7.82-7.90 (m, 2H, arom), 7.53-76 (m, 3H, arom), 7.41 (brs, 1H, =CH), 7.23 (m, 2H, arom), 6.82-6.90 (m, 2H, arom), 5.72 (dd, *J* = 11.6, 5.6 Hz 1H, C$\underline{H_2}$CH), 4.17 (t, 2H, *J* = 7.3 Hz, CH$_2$), 4.01 (q, 2H, *J* = 7.1 Hz, CH$_2$), 3.88 (dd, *J* = 18.3, 11.9 Hz, 1H, C$\underline{H_2}$CH), 3.52 (dd, *J* = 17.9, 6.1 Hz, 1H, CH$_2$C$\underline{H}$,), 2.60 (t, 2H, *J* = 7.1Hz, CH$_2$), 1.14 (t, 3H, *J* = 7.1Hz, CH$_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.7, 170.8, 166.2, 160.6, 155.8, 133.8, 131.709, 130.6, 130.6, 129.7, 129.6,

127.7, 125.5, 119.8, 116.5, 93.0, 62.6, 60.8, 50.0, 40.7, 31.6, 14.4. LCMS (ESI+) *m/z* 482 $(M+H)^+$. Calcd. for $C_{24}H_{23}N_3O_4S_2$: C, 59.86; H, 4.81; N, 8.73; Found: C, 60.00; H, 5.00; N, 8.60%.

4.1.2.8. (Z)-5-[5-(4-Dimethylaminophenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one (**8**). Spectral and analytical data are described [51].

4.1.2.9. (Z)-5-[5-(4-Dimethylaminophenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-2-thioxothiazolidin-4-one (**9**). Spectral and analytical data are described [51].

4.1.2.10. (Z)-5-[3,5-Di-(4-chlorophenyl)-4,5-dihydropyrazol-1-ylmethylene]-2-thioxothiazolidin-4-one (**10**). Yield 72%, mp 289-291 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.95 (s, 1H, NH), 7.83 (d, 2H, *J* = 8.4 Hz, arom), 7.65 (d, 2H, *J* = 8.6 Hz, arom), 7.51 (d, 2H, *J* = 8.4 Hz, arom), 7.41 (d, 2H, *J* = 8.4 Hz, arom), 7.32 (s, 1H, =CH), 5.65 (dd, 1H, *J* = 11.1, 6.2 Hz, C$\underline{H_2}$CH), 4.00 (dd, 1H, *J*= 18.2, 11.9 Hz, C$\underline{H_2}$CH), 3.46 (dd, 1H, *J* = 18.3, 5.8 Hz, $CH_2C\underline{H}$). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 195.9, 168.2, 157.5, 138.9, 135.6, 133.4, 132.3, 129.2, 129.1, 128.9, 128.8, 97.1, 64.2, 41.4. LCMS (ESI+) *m/z* 434/436/438 $(M+H)^+$. Calcd. for $C_{19}H_{13}Cl_2N_3OS_2$: C, 52.54; H, 3.02; N, 9.67; Found: C, 52.70; H, 3.20; N, 9.80%.

4.1.2.11. (Z)-5-[3,5-Di-(4-chlorophenyl)-4,5-dihydropyrazol-1-ylmethylene]-3-(4-acetoxyphenyl)-2-thioxothiazolidin-4-one (**11**). Yield 70%, mp 284-286 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.85 (d, 2H, *J* = 8.1 Hz, arom), 7.67 (d, 2H, *J* = 7.9 Hz, arom), 7.55 (s, 1H, =CH), 7.52 (d, 2H, *J* = 7.9 Hz, arom), 7.42 (d, 2H, *J* = 8.0 Hz, arom), 7.31 (d, 2H, *J* = 8.0 Hz, arom), 7.25 (d, 2H, *J* = 8.2 Hz, arom), 5.74 (dd, 1H, *J* = 10.6, 5.6 Hz, C$\underline{H_2}$CH), 4.05 (dd, 1H, *J* = 17.4, 10.8 Hz, C$\underline{H_2}$CH), 3.51 (dd, 1H, *J* = 16.8, 5.3 Hz, $CH_2C\underline{H}$), 2.29 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 194.9, 169.6, 166.7, 158.9, 151.0, 136.4, 134.0, 133.9, 133.825, 130.5, 129.8, 129.5, 129.4, 129.3, 129.2, 123.0, 108.4, 95.0, 64.8, 41.9, 21.4. LCMS (ESI+) *m/z* 568/570/572 $(M+H)^+$. Calcd. for $C_{27}H_{19}Cl_2N_3O_3S_2$: C, 57.04; H, 3.37; N, 7.39; Found: C, 57.20; H, 3.30; N, 7.20%.

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4.1.2.12. (Z)-5-[5-(2-Hydroxyphenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-ylmethylene]-3-(4-acetoxyphenyl)-2-thioxothiazolidin-4-one (**12**). Yield 79%, mp 241-242 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.04 (s, 1H, OH), 8.32 (s, 1H, arom), 8.12 (brs, 2H, arom), 8.00-8.08 (m, 3H, arom), 7.98 (d, 2H, *J* = 8.8 Hz, arom), 7.52-7.66 (m, 3H, arom), 7.50 (s, 1H, =CH), 7.32 (d, 2H, *J* = 8.7 Hz, arom), 7.21-7.30 (m, 2H, arom), 6.90 (d, 1H, *J* = 8.3 Hz, arom), 6.87 (t, 1H, *J* = 7.0 Hz, arom), 5.81 (dd, 1H, *J* = 11.5, 6.0 Hz, C$\underline{H_2}$CH), 4.02 (dd, 1H, *J* = 17.3, 11.6 Hz, C$\underline{H_2}$CH), 3.66 (dd, 1H, *J* = 18.2, 5.5 Hz, CH$_2$C$\underline{H}$), 2.30 (s, 3H, CH$_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.9, 168.9, 165.9, 159.9, 155.2, 150.4, 133.8, 133.4, 133.12, 132.6, 129.9, 128.9, 127.8, 127.4, 127.0, 126.7, 126.4, 125.0, 123.64, 122.9, 122.4, 119.3, 115.9, 93.3, 62.1, 41.0, 20.9. LCMS (ESI+) *m/z* 566 $(M+H)^+$. Calcd. for $C_{31}H_{23}N_3O_4S_2$: C, 65.82; H, 4.10; N, 7.43; Found: C, 66.00; H, 4.30; N, 7.20%.

4.1.2.13. (Z)-5-[5-(4-Chlorophenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-ylmethylene]-3-(4-acetoxyphenyl)-2-thioxothiazolidin-4-one (**13**). Yield 80%, mp 283-285 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.30 (s, 1H, arom), 8.11 (brs, 2H, arom), 7.98-8.03 (m, 3H, arom), 7.58-7.62 (m, 2H, arom), 7.52 (d, 2H, *J* = 7.5 Hz, arom), 7.44 (d, 2H, *J* = 8.0 Hz, arom), 7.33 (d, 2H, *J* = 8.5 Hz, arom), 7.26 (d, 2H, *J* = 8.0 Hz, arom), 5.79 (dd, 1H, *J* = 10.6, 5.5 Hz, C$\underline{H_2}$CH), 4.17 (dd, 1H, *J* = 17.3, 10.9 Hz, C$\underline{H_2}$CH), 3.63 (dd, 1H, *J* = 17.3, 5.5 Hz, CH$_2$C$\underline{H}$), 2.29 (s, 3H, CH$_3$). ^{13}C NMR (100 MHz, DMSO-*d*6): δ 194.8, 169.6, 166.7, 159.9, 151.0, 145.1, 139.8, 134.5, 134.1, 133.9, 133.8, 133.2, 130.5, 129.8, 129.4, 129.3, 129.2, 128.5, 128.4, 127.9, 127.7, 123.0, 94.9, 64.7, 42.0, 21.4. LCMS (ESI+) *m/z* 584/586 $(M+H)^+$. Calcd. for $C_{31}H_{22}ClN_3O_3S_2$: C, 63.74; H, 3.80; N, 7.19; Found: C, 63.90; H, 4.00; N, 7.10%.

4.1.2.14. (Z)-5-[5-(4-Methoxyphenyl)-3-(naphthalen-2-yl)-4,5-dihydropyrazol-1-ylmethylene]-3-(4-acetoxyphenyl)-2-thioxothiazolidin-4-one (**14**). Yield 80%, mp 256-258 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.32 (s, 1H, arom.), 8.13 (brs, 2H, arom.), 7.99-8.05 (m, 2H, arom.), 7.60-76.3 (m, 2H, arom.), 7.50 (s, 1H. =CH), 7.38 (d, *J* = 7.8 Hz, 2H, arom.), 7.32 (d, *J* = 7.8 Hz, 2H, arom.), 7.26 (d, *J* = 8.5 Hz, 2H, arom.), 7.01 (d, 2H, *J* = 8.5 Hz, arom.), 5.70 (m, 1H, C$\underline{H_2}$CH), 4.14 (dd, 1H, *J* = 18.5, 11.0 Hz, C$\underline{H_2}$CH), 3.77 (s, 3H, OCH$_3$), 3.64 (dd,1H, *J* = 19.0, 6.0 Hz, CH$_2$C$\underline{H}$), 2.30 (s, 3H, CH$_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.3, 169.2, 166.2, 159.8, 159.7, 150.6, 144.3, 134.1, 133.5, 132.8, 132.0,

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130.1, 129.0, 128.90, 128.8, 128.6, 128.0, 128.0, 127.6, 127.2, 123.1, 122.6, 114.8, 94.0, 64.7, 55.3, 41.5, 21.0. LCMS (ESI+) *m/z* 580 $(M+H)^+$. Calcd. for $C_{32}H_{25}N_3O_4S_2$: C, 66.30; H, 4.35; N, 7.25; Found: C, 66.50; H, 4.50; N, 7.10%.

4.1.2.15. (Z)-5-[5-(2-Hydroxyphenyl)-3-(naphthalen-2-yl)-4,5-dihydropyrazol-1-ylmethylene]-3-methyl-2-thioxothiazolidin-4-one (**15**). Yield 75%, mp 280-282 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.95 (s, 1H, OH), 8.28 (brs, 1H, arom.), 8.21-8.24 (m, 1H, arom), 8.09 (brs, 1H, arom), 7.93-8.06 (m. 2H, arom), 7.56-7.59 (m, 2H, arom), 7.46 (s, 1H, =CH), 7.20-7.25 (m, 2H, arom), 6.91 (d, 1H, *J* = 7.6 Hz, arom), 6.86 (t, 1H, *J* = 6.4 Hz, arom), 5.78 (dd, 1H, *J* = 11.2, 6.7 Hz, C$\underline{H_2}$CH), 4.03 (dd, 1H, *J* = 18.0, 11.0 Hz, C$\underline{H_2}$CH), 3.64 (dd,1H, *J* = 18.2, 6.4 Hz, $CH_2\underline{CH}$), 2.45 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.9, 166.6, 158.0, 155.8, 135.8, 134.1, 133.3, 130.6, 129.2, 128.3, 128.0, 127.6, 127.3, 127.0, 125.6, 124.2, 123.5, 119.9, 116.5, 93.7, 62.6, 15.2,. LCMS (ESI+) *m/z* 446 $(M+H)^+$. Calcd. for $C_{24}H_{19}N_3O_2S_2$: C, 64.70; H, 4.30; N, 9.43; Found: C, 64.90; H, 4.10; N, 9.30%.

4.1.2.16. (Z)-5-[5-(2-Hydroxyphenyl)-3-(naphthalen-2-yl)-4,5-dihydropyrazol-1-ylmethylene]-3-(piridin-3-yl)-2-thioxothiazolidin-4-one (**16**). Yield 81%, mp 260-262 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.05 (s, 1H, OH), 8.64 (m, 1H, arom), 8.52 (s, 1H, arom), 8.42 (s, 1H, arom), 8.33 (s, 1H, arom), 8.23 (d, 1H, *J* = 8.7 Hz, arom), 8.13 (brs, 2H, arom), 7.91-8.08 (m, 4H, arom), 7.82 (d, 2H, *J* = 7.5 Hz, arom), 7.58-7.65 (m, 4H, arom), 7.53 (s, 1H, =CH), 7.23-7.29 (m, 2H, arom), 6.90 (d, 1H, *J* = 7.8 Hz, arom), 6.87 (t, 1H, *J* = 7.3 Hz, arom), 5.75 (dd, 1H, *J* = 11.0, 6.5 Hz, C$\underline{H_2}$CH), 4.03 (dd, 1H, *J* = 17.5, 11.5 Hz, C$\underline{H_2}$CH), 3.68 (dd, 1H, *J* = 17.6, 6.4 Hz, $CH_2C\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.9, 165.7, 160.2, 155.2, 149.5, 149.4, 136.8, 133.9, 133.4, 132.9, 132.6, 130.1, 129.0, 128.7, 128.6, 127.8, 127.5, 127.0, 124.9, 123.9, 122.9, 119.3, 115.9, 93.2, 62.2, 41.1 LCMS (ESI+) *m/z* 509 $(M+H)^+$. Calcd. for $C_{28}H_{20}N_4O_2S_2$: C, 66.12; H, 3.96; N, 11.02; Found: C, 66.30; H, 4.20; N, 10.90%.

4.1.2.17. (Z)-5-[5-(4-Chlorophenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one (**17**). Spectral and analytical data are described [51].

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4.1.2.18. (Z)-5-[5-(4-Chlorophenyl)-3-(naphthalen-2-yl)-4,5-dihydropyrazol-1-ylmethylene]-2-thioxothiazolidin-4-one (**18**). Yield 81%, mp 283-285 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.96 (s, 1H, NH), 8.26 (s, 1H, arom), 8.09 (d, 1H, *J* = 8.7 Hz, arom), 8.06 (d, 1H, *J* = 8.9 Hz, arom), 8.00 (t, 2H, *J* = 8.9 Hz, arom), 7.59-7.64 (m, 2H, arom), 7.52 (d, 2H, *J* = 8.3 Hz, arom), 7.44 (d, 2H, *J* = 8.3 Hz, arom), 7.37 (s, 1H, arom), 5.73 (dd, 1H, *J* = 11.5, 6.3 Hz, C$\underline{H_2}$CH), 4.13 (dd, 1H, *J* = 18.2, 11.4 Hz, C$\underline{H_2}$CH), 3.59 (dd, 1H, *J* = 18.1, 6.1 Hz, CH$_2$C$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 195.8, 168.2, 162.2, 158.6, 139.1, 133.8, 133.3, 132.5, 132.4, 129.2, 128.9, 128.6, 128.6, 127.8, 127.4, 127.0, 122.9, 96.9, 64.1, 41.5. LCMS (ESI+) *m/z* 450/452 $(M+H)^+$. Calcd. for $C_{23}H_{16}ClN_3OS_2$: C, 61.39; H, 3.58; N, 9.34; Found: C, 61.50; H, 3.70; N, 9.20%.

4.1.2.19. (Z)-5-[5-(2-Hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-4-thioxothiazolidin-2-one (**19**). Spectral and analytical characteristics are described [51].

4.1.2.20. (Z)-5-[5-(4-Chlorophenyl)-3-(naphtalen-2-yl)-4,5-dihydropyrazol-1-ylmethylene]-4-thioxothiazolidin-2-one (**20**). Yield 82%, mp 293-285 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.52 (s, 1H, NH), 8.18 (s, 1H, =CH), 8.05 (d, 1H, *J* = 8.5 Hz, arom), 7.91-7.97 (m, 4H, arom.), 7.52-7.58 (m, 2H, arom), 7.40-7.48 (m, 4H, arom), 5.82 (dd, 1H, *J* = 10.9, 5.9 Hz, C$\underline{H_2}$CH), 4.12 (dd, 1H, *J* = 17.9, 11.5 Hz, C$\underline{H_2}$CH), 3.53 (dd, 1H, *J* = 18.0, 5.9 Hz, CH$_2$C$\underline{H}$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 187.7, 172.9, 160.6, 139.4, 137.5, 134.6, 134.1, 133.1, 129.9, 129.7, 129.6, 129.3, 129.2, 128.5, 128.4, 127.8, 127.7, 123.8, 105.7, 65.3, 41.5. LCMS (ESI+) *m/z* 450/452 $(M+H)^+$. Calcd. for $C_{23}H_{16}ClN_3OS_2$: C, 61.39; H, 3.58; N, 9.34; Found: C, 61.50; H, 3.60; N, 9.10%.

4.1.3. Crystal structure determination of 5-[5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one (**1**).

Crystal Data: $C_{27}H_{21}N_3O_4S_2$, 0.75(C_2H_6O), M_r = 550.14, triclinic, space group *P*-1, *a* = 11.41562(10) Å, *b* = 15.6249(2) Å, *c* = 29.8642(4) Å, *α* = 88.0515(11)°, *β* = 86.1259(9)°, *γ* = 78.3790(9)°, *V* = 5204.56(11) Å^3, *Z* = 8, D_{calc} = 1.404 /cm^3, *μ* = 2.226 mm^{-1}, *T* = 130(2) K.

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Data Collection: A yellow plate crystal (CH_3CH_2OH) of 0.16 mm × 0.08 mm× 0.05 mm was used to record 52573 (Cu *K*α radiation, θ_{max} = 76.5°) intensities on an Agilent SuperNova four-circle diffractometer equipped with an Atlas CCD detector (CrysAlis PRO, Version 1.171.37.35; Agilent Technologies Ltd. (Oxford Diffraction): Yarnton, UK, 2014) using mirror monochromatized Cu *K*α radiation from a high-flux microfocus source (λ = 1.54178 Å). Accurate unit cell parameters were determined by least-squares techniques from the θ values of 25621 reflections, θ range 2.9−76.0°. The data were corrected for Lorentz polarization and for absorption effects (CrysAlis PRO, Version 1.171.37.35; Agilent Technologies Ltd. (Oxford Diffraction): Yarnton, UK, 2014). The 20510 total unique reflections (R_{int} = 0.0304) were used for structure determination.

Structure Solution and Refinement. The structure was solved by direct methods (SHELXS-97), and refined against F^2 for all data (SHELXL-97) [76]. The positions of the H atoms bonded to O36A, O36B, O36C and O36D atoms were obtained from the difference Fourier maps and were refined freely. The remaining H atoms were placed geometrically in calculated positions and were refined with a riding model, with C−H = 0.98 Å (CH_3), 0.99 Å (CH_2), 1.00 Å ($C_{sp}3H$), 0.95 Å ($C_{ar}H$) and $U_{iso}(H) = 1.2U_{eq}(C)$ or $1.5U_{eq}(C)$ for methyl H atoms. The methyl groups were refined as rigid groups, which were allowed to rotate. Final refinement converged with R = 0.0520 (for 17462 data with $F^2 > 4\sigma(F^2)$), wR = 0.1382 (on F^2 for all data), and S = 1.068 (on F^2 for all data). The largest difference peak and hole was 0.649 and -0.480 e $Å^{-3}$. The molecular illustrations were drawn using ORTEP-3 for Windows [77].

Crystallographic data for *(Z)-5-[5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one* have been deposited at the Cambridge Crystallographic Data Centre (CCDC), 12 Union ROAD, Cambridge CB2 1EZ (U.K.) (phone, (+44) 1223/336-408; fax, (+44) 1223/336-033; e-mail, deposit@ccdc.cam.ac.uk; World Wide Web, http://www.ccdc.cam.ac.uk (deposition no. **CCDC 1432661**)).

4.2. Biological experiments

4.2.1. Materials

Stock solutions of 4-thiazolidones (10 mM) were prepared in the dimethyl sulfoxide (Sigma-Aldrich, USA), and additionally dissolved in culture medium prior to addition to the cell culture. 3-(4,5-

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Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) (99.5% pure), DMEM, N-acetyl-cysteine (NAC), ascorbic acid (AA), propidium iodide (PI), Hoechst-33258 and FITC-conjugated Annexin Vwere obtained from Sigma-Aldrich, USA; RPMI and fetal bovine serum were obtained from APP, Austria. Doxorubicin was purchased from Pharmachemie B.V., the Netherlands.

4.2.2. Cell cultures

Human promyelocytic leukemia cells of HL-60 line, adriamycin-resistant cells of HL-60/ADR line with over-expression of P-glycoprotein, and human ovarian carcinoma cells of Skov3 line were obtained from the American type culture collection (ATCC, Minnesota, USA). Human acute T-cell leukemia cells of Jurkat line, mouse lymphocytic leukemia cells of L1210 line, human breast adenocarcinoma cells of MCF-7 line, transformed mouse fibroblasts of L929 line, human embryonic kidney cells of HEK293T line, and murine mesenchymal preosteoblastic cells of KS 483 line were obtained from Collection at R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiology (Kyiv, Ukraine). Cells were grown in the RPMI or DMEM culture medium supplemented with 10% fetal bovine serum. Cells were cultivated in the CO_2-thermostate at 37^{o}C in atmosphere of 95% air and 5% CO_2.

4.2.3. Cytotoxicity assays

In vitro screening of anticancer activity of the synthesized compounds and doxorubicin used as a reference drug control towards cancer cell lines was measured by the MTT test [78]. Tumor cells were seeded for 24 hours in 96-well microtiter plates at a concentration of 2,000 substrate-dependent cells/well or 10,000 suspension cells/well (100 μL/well), after that cells were incubated for 72 hours with various additions of the synthesized compounds (0; 0.01; 0.1; 0.5; 1.0; 10.0 μM). MTT which is converted to dark blue, water insoluble MTT formazan by the mitochondrial dehydrogenases, was used to determine viable cells according to the manufacturer's protocol (Sigma-Aldrich, USA). The IC_{50} of tested compounds was calculated as a lethal concentration of drug killing 50 % cells in comparison with an untreated culture.

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For estimating the impact of the antioxidants (AA and NAC) on the cytotoxic activity of 4-thiazolidinones, different doses of drugs were used in combination with cell pretreatment for 30 min with the non-toxic doses of the antioxidants (NAC at 1.0; 2.0 mM and AA at 25.0; 100.0 μM).

4.2.4. Fluorescent microscopy

Duplicate slides were prepared for each cell type/treatment group and >500 cells were counted in each sample. FITC-conjugated Annexin V and PI double staining were performed in order to detect early apoptotic events in HL-60 cells treated with 4-thiazolidinones. In 48 h after the addition of the tested compound, the HL-60 cells were pelleted by centrifugation at 2,000 rpm, washed twice with 1x PBS, and incubated for 15 min in the Annexin V binding buffer containing 1/20 volume of FITC-conjugated Annexin V solution and PI (20.0 μg/mL). 10 μL of cell suspension were added on slides and cover glasses were placed over them. Cells were examined under Zeiss AxioImager A1 fluorescent microscope (Carl Zeiss, Germany).

4.2.5. Western blot analysis

After 48 h exposure of the tested compound, cell proteins were isolated, resolved by the SDS/PAGE, and transferred onto a polyvinylidenedifluoride (PVDF) membrane for Western blotting, as described [60]. The following antibodies were used in this study at a 1:1.000 dilution: anti-p38, anti-phospho-ERK ½ (Thr 202/Tyr 204), anti-EndoG, anti-phospho-STAT3 (Tyr 705), anti-p21 (Waf1/Cip1), anti-phospho-cdc2 (Tyr 15) (Cdk1), anti-phospho-Rb (Ser 807/811) (Cell Signaling Technology, USA), anti-Bax (sc-6236), anti-Bcl-XL/S (sc-634), anti-JNK (sc-571), anti-CyclinD3 (C-16, sc-182) (Santa Cruz Biotec, USA). Equal loading of each lane was evaluated by the immunoblotting of the same membrane with the anti-beta-actin monoclonal mouse AC-15 (Sigma-Aldrich, USA). All secondary peroxidase-labelled antibodies (Cell Signaling, USA) were used at working dilution of 1:5,000.

4.2.6. Cell cycle analysis

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HL-60 cells were seeded into 6-well plates and then treated for 8 and 48 h with the tested compounds at different concentrations. To analyze cell cycle distribution, cells were collected, washed with the PBS, fixed with 70% ethanol, and stored at –20°C. For analysis, cells were transferred in the PBS, incubated with RNAse (10 μg/ml) for 30 min at 37°C, treated for 30 min with propidium iodide (5 μg/ml), and analyzed by flow cytometry (Becton Dickinson, Palo Alto, CA).

4.2.7. Statistical analysis

All data are presented as the mean ± standart deviation (SD). Results were analyzed and illustrated with GraphPad Prism (version 5; GraphPad Software, San Diego, CA). Statistical analyses were performed using two-way ANOVA with Bonferroni post-tests (apoptosis induction, tumor growth). P-value of <0.05 was considered as statistically significant.

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Supplementary data

The representative ^{1}H and ^{13}C NMR spectra of synthesized compound are presented.

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Figure 1. Synthesis of 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-2-thioxothiazolidin-4-ones and 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-4-thioxothiazolidin-2-ones. Reagents, reaction conditions: (a) $CH(OEt)_3$, Ac_2O, reflux 1 h; (b) appropriate amine, EtOH, reflux 1 h.

Figure 2. View of the symmetry-independent molecule B of compound **1**. The atomic labelling scheme.

Figure 3. Two views of symmetry independent molecules A – D for compound **1**. The deviation of 2-hydroxyphenyl and 3-acetoxyphenyl moieties from the mean plane of 2-thioxothiazolidin-4-one system.

Figure 4. Cytotoxicity of compound **1** towards different cell lines. After a total experimental time (72 h), cell vitality was detected by the MTT assay.

Figure 5. Exposure time-dependency of compound **1** cytotoxicity. Pulsing experiments were performed on HL-60 and HL-60/ADR leukemia cells after 3, 6, 24, 48 and 72 h of compound **1** exposition. Viability of cells was determined using MTT assay.

Figure 6. Protective effect of N-acetylcysteine (NAC) and ascorbic acid (AA) on the cytotoxic action of compound **1** towards HL-60 and HL-60/ADR cells. Compound **1** was added to the cells after 30 min

Figure 7. Expression of the apoptotic proteins in HL-60 leukemia cells after 48 h of compound **1** treatment (**A**); ratio of dead cells after 48 h treatment test compound (**B**). Ratio of dead cells was determined by the Annexin V/PI co-staining in HL-60 cells. The percentage of apoptotic cells was analyzed by the fluorescent microscopy study of the Annexin V/PI double staining of HL-60 cells treated with compound **1** for 48 h.

Figure 8. Results of induction of the G_0/G_1 arrest by compound **1** (**A**); expression of the indicated cell cycle regulatory proteins in HL-60 cells after 48h of compound **1** treatment (**B**). Impact on cell cycle distribution was determined in HL-60 cells after 8 and 48 h drug treatment by PI-staining and flow cytometry.

Table 1. Antiproliferative activity of tested compounds.

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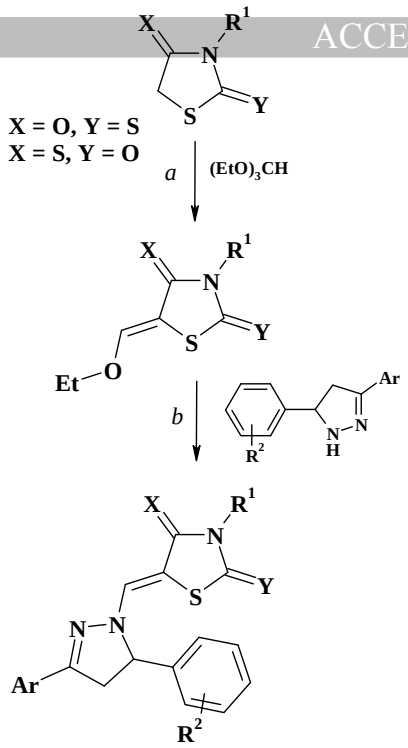

	X	Y	R¹	R²	Ar
1	O	S	3-AcO-C_6H_4	2-OH	Ph
2	O	S	4-AcO-C_6H_4	2-OH	Ph
3	O	S	Me	2-OH	Ph
4	O	S	Et	2-OH	Ph
5	O	S	pyridin-3-yl	2-OH	Ph
6	O	S	3-CF_3-C_6H_4	2-OH	Ph
7	O	S	$(CH_2)_2$COOEt	2-OH	Ph
8	O	S	3-AcO-C_6H_4	4-Me_2N	Ph
9	O	S	H	4-Me_2N	Ph
10	O	S	H	4-Cl	4-C_6H_4
11	O	S	4-AcO-C_6H_4	4-Cl	4-C_6H_4
12	O	S	4-AcO-C_6H_4	2-OH	naphthalen-2-yl
13	O	S	4-AcO-C_6H_4	4-Cl	naphthalen-2-yl
14	O	S	4-AcO-C_6H_4	4-OMe	naphthalen-2-yl
15	O	S	Me	2-OH	naphthalen-2-yl
16	O	S	pyridin-3-yl	2-OH	naphthalen-2-yl
17	O	S	3-AcO-C_6H_4	4-Cl	naphthalen-2-yl
18	O	S	H	4-Cl	naphthalen-2-yl
19	S	O	H	2-OH	Ph
20	S	O	H	4-Cl	naphthalen-2-yl

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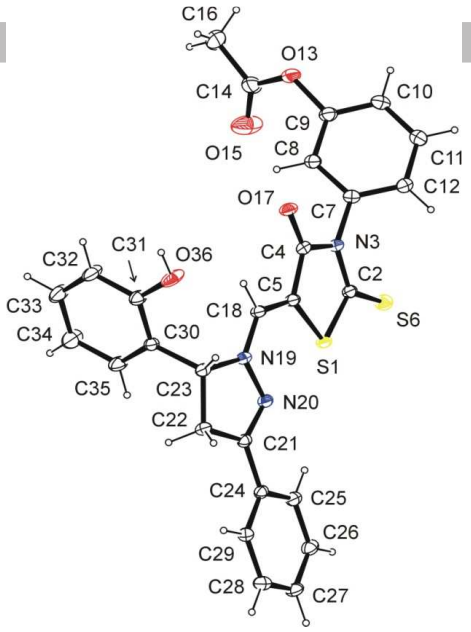

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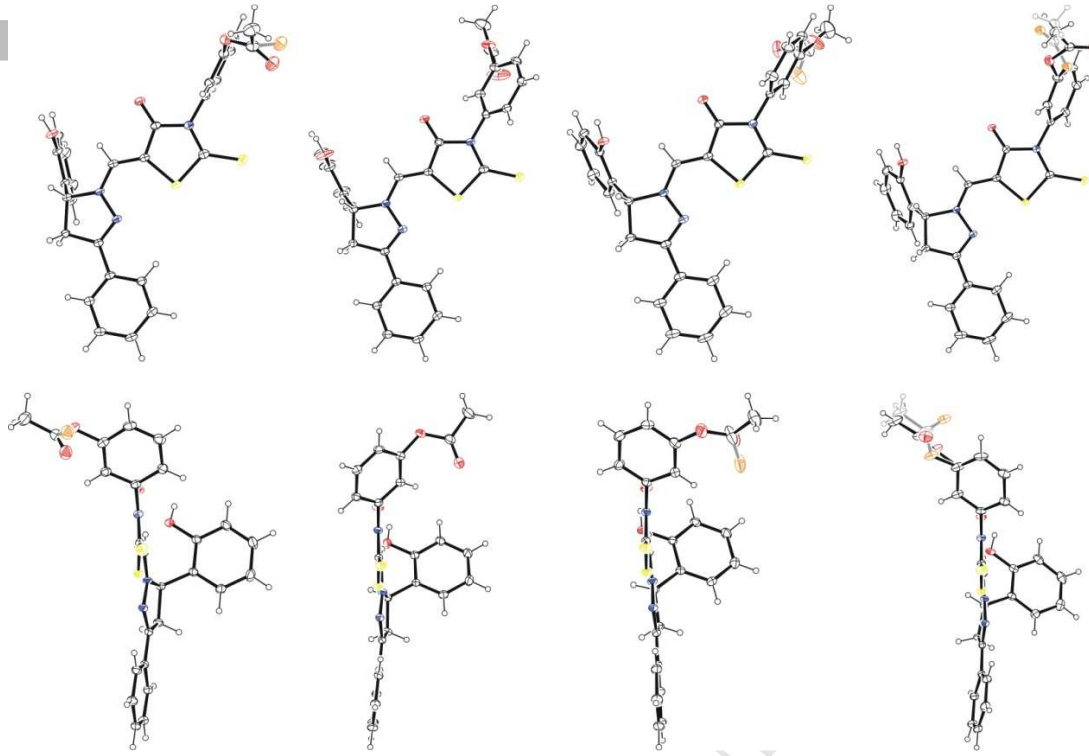

A **B** **C** **D**

Figure 3. Two views of symmetry independent molecules A – D for compound **1**. The deviation of 2-hydroxyphenyl and 3-acetoxyphenyl moieties from the mean plane of 2-thioxothiazolidin-4-one system.

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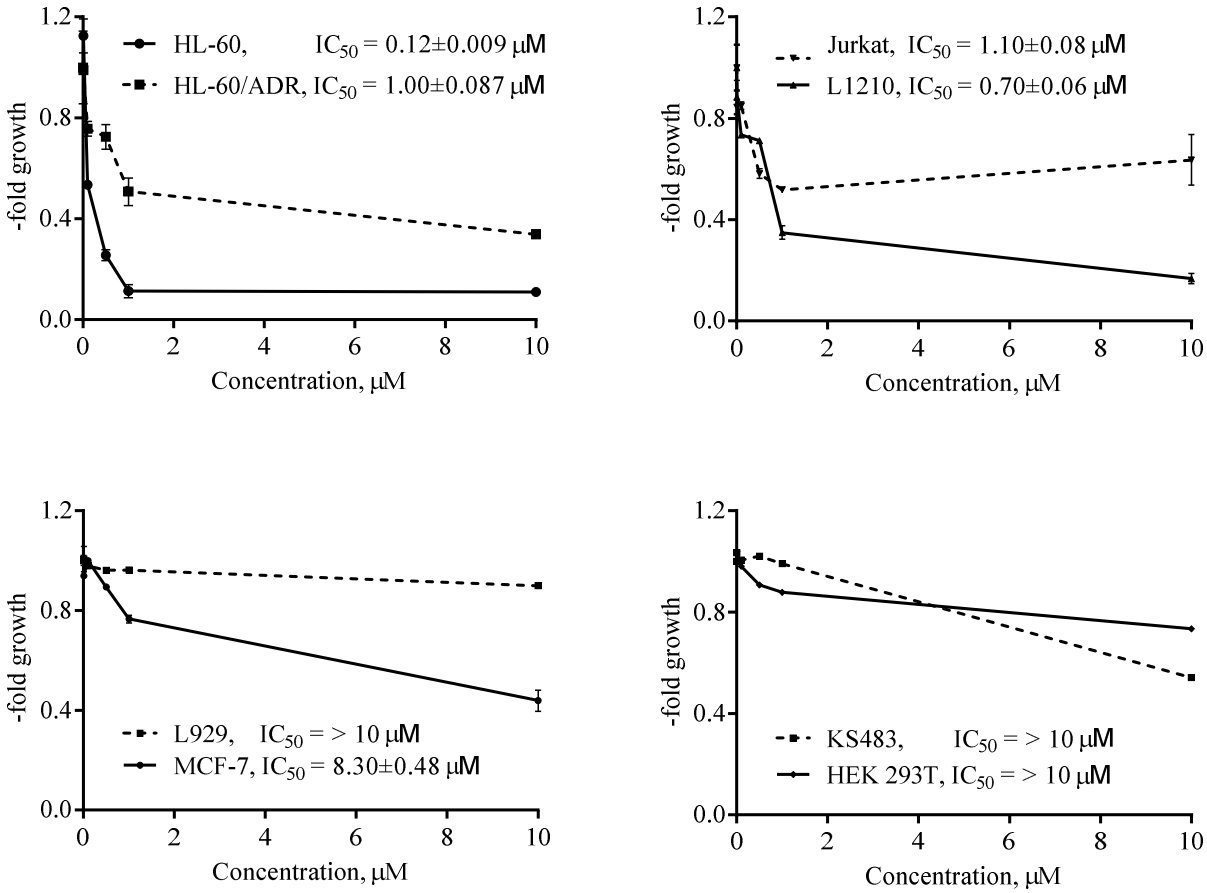

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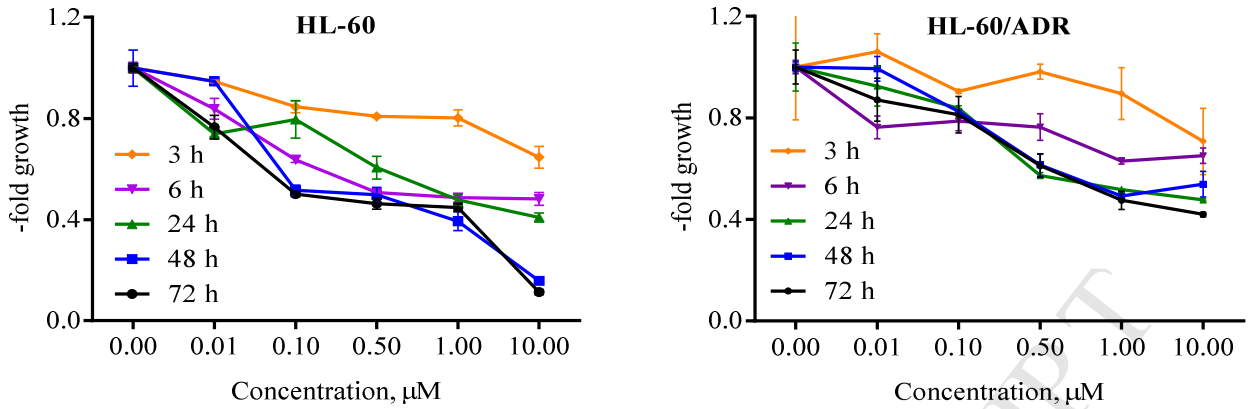

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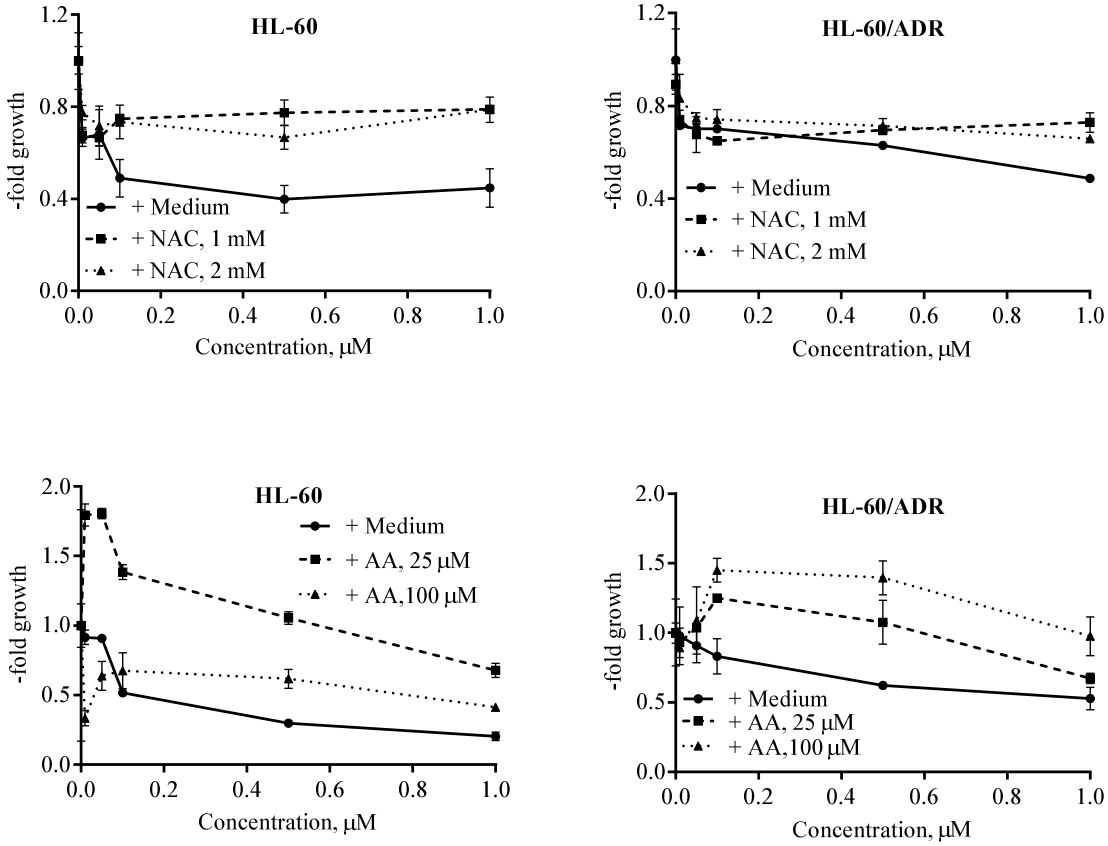

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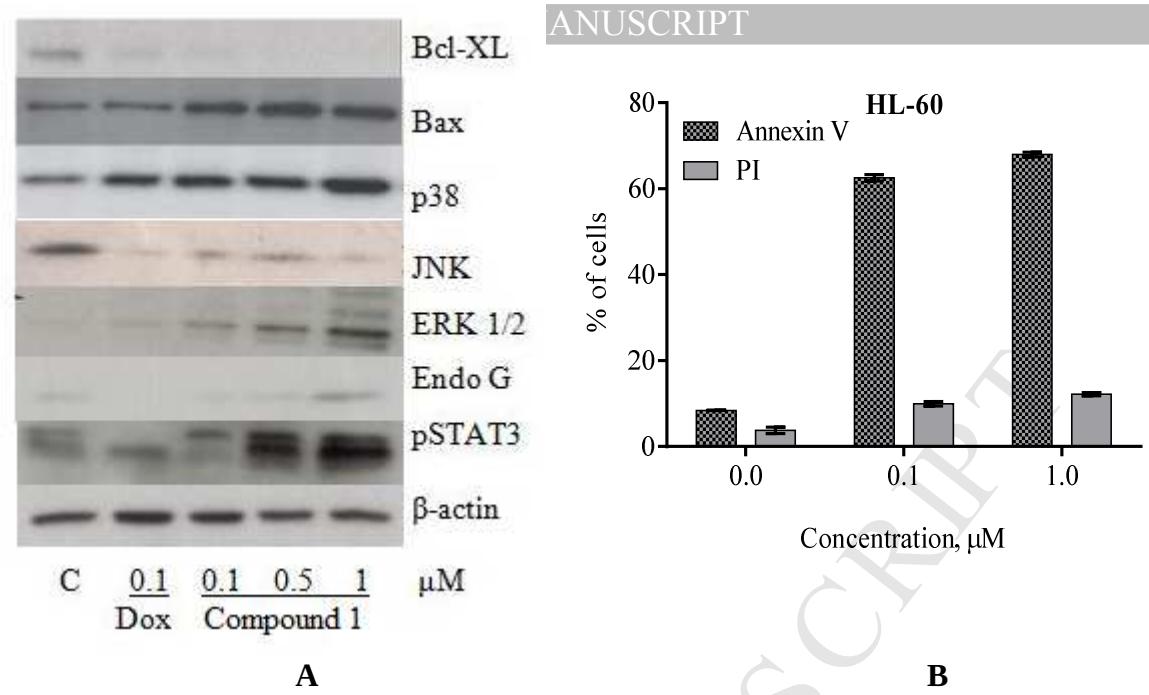

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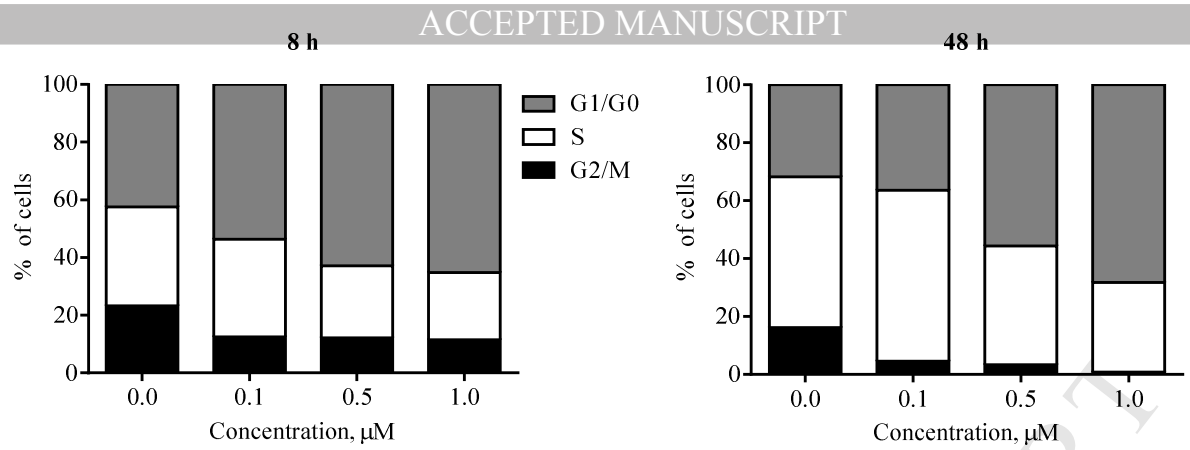
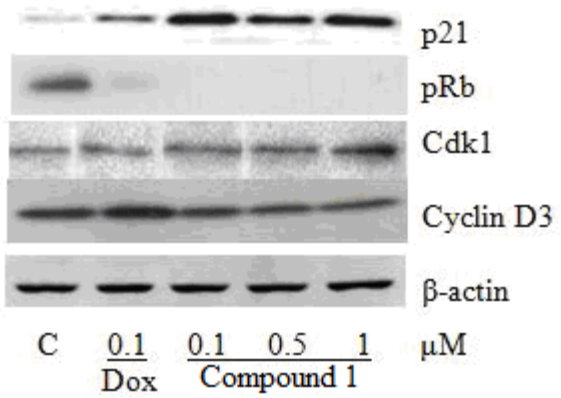


(A)



(B)

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Table 1. Antiproliferative activity of tested compounds

Compound	IC_{50}, μM	
	HL-60	**Skov3**
1	0.118±0.009	> 10
2	9.400±2.040	0.244±0.020
3	2.880±0.192	0.341±0.026
4	> 10	> 10
5	0.720±0.013	> 10
6	> 10	> 10
7	> 10	> 10
8	0.100±0.009	> 10
9	> 10	> 10
10	> 10	9.143±0.163
11	> 10	> 10
12	0.500±0.046	> 10
13	1.800±0.030	> 10
14	> 10	1.103±0.039
15	> 10	> 10
16	4.060±0.260	5.589±0.380
17	1.020±0.091	2.390±0.113
18	0.400±0.052	0.163±0.009
19	0.990±0.087	7.87±0.094
20	0.156±0.014	> 10
Dox	0.051±0.003	0.073±0.006

IC_{50} is presented as a mean ± SD of three independent experiments performed in triplicate.

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Highlights

for "**5-Ene-4-thiazolidinones induce apoptosis in mammalian leukemia cells**"

by Julia Senkiv, Nataliya Finiuk, Danylo Kaminskyy, Dmytro Havrylyuk, Magdalena Wojtyra, Iryna Kril, Andrzej Gzella, Rostyslav Stoika, Roman Lesyk

- New 5-enamine-4-thiazolidinones with selective antileukemic action.
- Mitochondria-depended apoptosis was identified as the main mode of action.
- Compound 1 induces G_0/G_1 arrest and activation of ROS production.
- Compound 1 possesses low toxicity towards pseudonormal cells

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