

# DRUG DEVELOPMENT

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## SYNTHESIS AND ANTI-LEUKEMIC ACTIVITY OF PYRROLIDINEDIONE-THIAZOLIDINONE HYBRIDS

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A series of novel 2-(5-ylidene-4-oxo-2-thioxo-thiazolidin-3-yl)-succinimides and 5-ylidene-3-(1-arylpyrrolidine-2,5-dione)-thiazolidine-2,4-diones were synthesized. An efficient simple protocol for rhodanine-pyrrolidinedione hybrids synthesis which allows avoiding the step of anhydride formation was proposed. Following the previous data on antileukemic properties of related thiazolidinone derivatives, the activity of 19 target compounds was investigated towards four leukemia cell lines: Dami, HL-60, Jurkat, and K562. Among the tested compounds, 3-[5-(4-chloro-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-1-phenyl-pyrrolidine-2,5-dione (Compound **1**) possessed good and selective antiproliferative action against Dami and HL-60 cell lines and satisfactory toxicity level (acute toxicity evaluated *in vivo* in mice).

**Key words:** 4-thiazolidinone, pyrrolidinedione, anticancer activity, anti-leukemic activity.

### Introduction

4-Thiazolidones are known for their good pharmacological profile, and anticancer activity is one of the most studied characteristics of this class of small heterocyclic molecules [1-5]. Different carboxylic acids/amides on the base of the 4-thiazolidinone core are well-known, and the methods of their synthesis are well described [4]. Despite the great variety of these carboxylic acids, the 5-ene-thiazolidinone-3-carboxylic acids and their amides on the base of different cores (2,4-thiazolidinedione, rhodanine and isorhodanine, 2-amino(imino)4-thiazolidinone) are likely to be the most promising in the search for novel biologically active compounds [1,6,7]. The most studied and described types of activity for 5-ene-thiazolidinone-3-carboxylic acids

and related substances are: antibacterial [8-12] and antifungal [13] activities, antiparasitic activity (e.g. antimalarial [14] and anti-trypanosomal [15]), and antidiabetic activities [16, 17]. A series of original articles was dedicated to the search for efficient anticancer agents among the 5-ene-4-thiazolidinone-3-carboxylic acids [1, 6, 7, 18-20]. Molecules bearing a non-fused 5-ene-4-thiazolidinone fragment do not belong to any “classic” type of anticancer agent [1, 6, 21, 22]. Although the data on possible modes of 5-ene-4-thiazolidinone action are very scarce, a great part of the research in this field testifies to their pro-apoptotic effect [23, 24]. For example, 4-thiazolidinone-3-carboxylic acid amides with a furan fragment showed significant cytotoxicity and selectivity towards leukemia cells and were conside-

red to act through the induction of apoptosis [25] as well as involving the PPAR signaling pathway [26-28]. One more probable mode of action involves PPAR antagonistic activity that represents a new promising direction for cancer treatment [28, 29]. 5-Ene-rhodanine-3-carboxylic acids are well known selective ligands to “anticancer” biotargets: 3-[5-(4-fluorobenzylidene)-rhodanin-3-yl]-benzoic acid selectively inhibited phosphatases JSP-1 (JNK-stimulating phosphatase-1) [30], and 5-benzylidene-rhodanine-3-alcanecarboxylic acids inhibited the protein-protein interaction of antiapoptotic proteins of the Bcl-2 and Bax family and their binding to the appropriate receptor domains [31-35].

The crucial impact of the C5-(ylid)ene fragment on the level of anti-cancer activity was confirmed by numerous studies [1-5]. But recently 5-ene-4-thiazolidinones (especially 5-ene-rhodanines – “ene\_rhod\_A”) are treated as frequent hitters or pan assay interference compounds (PAINS) that may easily bind to various proteins, and thus possess low selectivity [36-38] in high throughput screening campaigns. This is due to possible Michael acceptor (MA) functionality that, however, often is not confirmed in experimental studies [39-41]. The PAINS concept remains controversial and such compounds should not be excluded from the drug development process *per se* [4]. Moreover, the possibility of targeting various proteins can also be considered as advantageous within the polypharmacological approach [37] or concept of multi-target drugs [42]. Such compounds being the examples of privileged scaffolds can be the structures that provide baseline affinity for a whole protein family [37, 43]. Additionally, the 5-ene-4-thiazolidinone scaffold offers great possibilities for molecular optimization that aims to increase selectivity and to transform it into other chemical classes to avoid functionalities of MA [4, 5, 44, 45].

Since there is a tendency of prevalent anti-leukemic action observed when analyzing the data on 5-ene-4-thiazolidinone anticancer activity [1, 2, 6, 25, 31, 46, 47], the study presented in this manuscript became the continuation of the search for small drug-like 4-thiazolidinone-3-carboxylic acids and their derivatives with anti-proliferative properties against leukemic cell lines.

## Materials and Methods

All reagents and materials were purchased from commercial sources and used without purifica-

tion. Melting points were measured in open capillary tubes on a BUCHI B-545 melting point apparatus (Flawil, Switzerland) and are uncorrected. The elemental analyses (C, H, N) were performed using the Perkin-Elmer 2400 CHN analyzer (Waltham, Massachusetts, USA) and were within  $\pm 0.4\%$  of the theoretical values. The  $^1\text{H}$  NMR spectra were recorded on Varian Gemini 400 MHz (Palo Alto, California, USA) and  $^{13}\text{C}$  NMR spectra on Varian Mercury-400 100 MHz in  $\text{DMSO}-d_6$  using tetramethylsilane as an internal standard. Chemical shifts are reported in ppm units with use of  $\delta$  scale.

### General procedure for 5-ylidene-3-(1-aryl-pyrrolidine-2,5-dione)-rhodanines synthesis (Compounds 1-6, 9, 10, 13-19)

*Method A.* A mixture of 5-ylidene-4-oxo-2-thioxothiazolidin-3-succinic acid (10 mmol) and 5 ml of thionyl chloride in 15 ml of a/h dioxane was refluxed for 1 h, cooled and precipitated by hexane. Formed anhydride was filtered off and used for further transformations without additional purification. A mixture of appropriate anhydride (5 mmol) and aromatic amine (5 mmol) in 10 ml of acetic acid was heated under reflux for 3 h. After cooling the reaction mixture, the obtained solid product was filtered off and recrystallized.

*Method B.* The mixture of 5-ylidene-rhodanine-3-succinic acid (5 mmol) and aromatic amine (5 mmol) in 10 ml of acetic acid was heated under reflux for 12-14 h. The progress of the reaction was monitored by TLC. After cooling, the formed precipitate was filtered off and recrystallized.

3-[5-(4-Chloro-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-1-phenyl-pyrrolidine-2,5-dione (Compound 1). Yield: 72% (method A), 65% (method B), mp 197-199 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  3.21-3.29 (m, 2H,  $\text{CH}_2$ ), 5.98, 6.27 (m, 1H,  $\text{CH}_2$ ), 7.26-7.31 (m, 2H, arom.), 7.47 (t, 1H,  $J = 6.8$  Hz, arom.), 7.53-7.56 (m, 2H, arom.), 7.64 (d, 2H,  $J = 7.6$  Hz, arom), 7.68-7.12 (m, 2H, arom.), 7.89, 7.96 (s, 1H,  $\text{CH}=\text{O}$ ).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  193.6, 174.3, 172.8, 166.7, 136.5, 133.9, 132.8, 132.1, 130.2, 129.6, 129.2, 127.3, 127.1, 122.1, 53.1, 33.0. Anal. calcd. for  $\text{C}_{20}\text{H}_{13}\text{ClN}_2\text{O}_3\text{S}_2$  (%): C 56.01, H 3.06, N 6.53; Found C 56.30, H 2.80, N 6.70.

4-{3-[5-(4-Chloro-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-2,5-dioxo-pyrrolidin-1-yl}-benzoic acid (Compound 2). Yield: 77% (method A), 55% (method B), mp 256-258 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  3.24-3.28 (m, 2H,  $\text{CH}_2$ ), 5.99 (dd,  $J = 6.6$  Hz, 9.5 Hz), 6.26-6.29 (m, 1H,  $\text{CH}_2$ ), 7.44

(d, 2H,  $J = 7.7$  Hz, arom.), 7.62 (d, 2H,  $J = 7.9$  Hz, arom.), 7.67 (d, 2H,  $J = 8.2$  Hz, arom.), 7.89, 7.96 (s, 1H, CH=), 8.11 (d, 2H,  $J = 8.2$  Hz, arom.), 12.85 (brs, 1H, COOH).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ),  $\delta$ , ppm: 193.6, 174.0, 172.6, 167.1, 166.8, 136.6, 136.4, 133.4, 132.9, 132.1, 131.4, 130.7, 130.2, 127.3, 122.1, 53.1, 33.1. Anal. calcd. for  $\text{C}_{21}\text{H}_{13}\text{ClN}_2\text{O}_5\text{S}_2$  (%): C 53.33, H 2.77, N 5.92; Found C 53.50, H 2.90, N 5.70.

*3-[5-(4-Chloro-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-1-(4-fluoro-phenyl)-pyrrolidine-2,5-dione (Compound 3)*. Yields: 66% (method A), 55% (method B), mp 226-228 °C.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.24-3.34 (m, 2H, CHCH $_2$ ), 5.91-5.94, 6.25 (m, 1H, CHCH $_2$ ), 7.32-7.38 (m, 2H, arom.), 7.40 (d, 2H,  $J = 8.2$  Hz, arom.), 7.64 (d, 2H,  $J = 7.7$  Hz, arom.), 7.69 (d, 2H,  $J = 8.4$  Hz, arom.), 7.89, 7.97 (s, 1H, CH).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  193.1, 173.8, 172.2, 163.4 (d,  $J = 273$  Hz), 161.8, 134.2, 133.7, 133.7, 133.2, 131.0, 129.6, 129.0, 120.1, 117.2 (d,  $J = 24$  Hz), 53.2, 33.1. Anal. calcd. for  $\text{C}_{20}\text{H}_{12}\text{ClFN}_2\text{O}_3\text{S}_2$  (%): C 53.75, H 2.71, N 6.27; Found C 53.60, H 2.50, N 6.40.

*3-[5-(4-Chloro-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-1-(4-chloro-phenyl)-pyrrolidine-2,5-dione (Compound 4)*. Yields: 72% (method A), 55% (method B), mp 255-256 °C.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.18-3.30 (m, 2H, CHCH $_2$ ), 5.83, 6.16 (m, 1H, CHCH $_2$ ), 7.56 (d, 2H,  $J = 8.8$  Hz, arom.), 7.63 (d, 2H,  $J = 8.8$  Hz, arom.), 7.66 (d, 4H, arom.), 7.79, 7.90 (s, 1H, CH).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  193.52, 173.6, 171.9, 162.0, 134.4, 133.7, 133.6, 133.2, 132.4, 131.0, 129.4, 128.7, 123.0, 116.4, 53.4, 32.9. Anal. calcd. for  $\text{C}_{20}\text{H}_{12}\text{Cl}_2\text{N}_2\text{O}_3\text{S}_2$  (%): C 51.84, H 2.61, N 6.05. Found C 52.00, H 2.70, N 5.90.

*4-{3-[5-(4-Chloro-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-2,5-dioxo-pyrrolidin-1-yl}-benzoic acid ethyl ester (Compound 5)*. Yields: 72% (method A), 51% (method B), mp 239-240 °C.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  1.33 (t, 3H,  $J = 7.0$  Hz, CH $_3$ ), 3.23-3.31 (m, 2H, CHCH $_2$ ), 4.28-4.30 (m, 2H, CH $_2$ CH $_3$ ), 5.75, 6.20 (m, 1H, CHCH $_2$ ), 7.52 (d, 2H,  $J = 8.1$  Hz, arom.), 7.63 (d, 2H,  $J = 8.2$  Hz, arom.), 7.78 (d, 2H,  $J = 7.9$  Hz, arom.), 7.82 (d, 2H,  $J = 8.1$  Hz, arom.), 7.90, 8.12 (s, 1H, CH).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ),  $\delta$ , ppm: 193.6, 173.9, 172.6, 166.8, 165.5, 136.5, 134.0, 133.4, 132.9, 132.8, 132.1, 130.5, 130.2, 127.4, 122.1, 61.5, 53.1, 33.0, 14.6. Anal. calcd. for  $\text{C}_{23}\text{H}_{17}\text{ClN}_2\text{O}_5\text{S}_2$  (%): C 55.14, H 3.42, N 5.59; Found C 55.40, H 3.50, N 5.40.

*3-[5-(4-Chloro-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-1-p-tolyl-pyrrolidine-2,5-dione*

*(Compound 6)*. Yields: 70% (method A), 58% (method B), mp 206-208 °C.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  2.40 (s, 3H, CH $_3$ ), 3.05 (dd,  $J = 18.5$  Hz,  $J = 5.9$  Hz), 3.16-3.35 (m, 2H, CHCH $_2$ ), 5.95, 6.25 (m, 1H, CHCH $_2$ ), 7.16 (d, 1H,  $J = 8.1$  Hz, arom.), 7.20 (d, 1H,  $J = 8.2$  Hz, arom.), 7.25-7.31 (m, 2H, arom.), 7.50-7.54 (m, 2H, arom.), 7.58-7.54 (m, 2H, arom.), 7.80, 7.89 (s, 1H, CH).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  192.6, 174.1, 173.1, 166.6, 136.4, 133.86, 132.9, 132.0, 130.4, 129.6, 129.2, 128.1, 127.4, 120.9, 53.1, 33.0, 21.4. Anal. calcd. for  $\text{C}_{21}\text{H}_{15}\text{ClN}_2\text{O}_3\text{S}_2$  (%): C 56.94, H 3.41, N 6.32; Found C 57.00, H 3.50, N 6.20.

*3-[5-(4-Fluoro-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-1-(3-trifluoromethyl-phenyl)-pyrrolidine-2,5-dione (Compound 9)*. Yield: 81% (method A), 74% (method B), mp 157-158 °C [7].  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.28-3.31 (m, 1H, CHCH $_2$ ), 3.43 (dd, 1H,  $J = 5.1$  Hz, 18.1 Hz, CHCH $_2$ ), 5.99 (dd,  $J = 5.1$  Hz, 8.8 Hz), 6.28 (m, 1H, CHCH $_2$ ), 7.42 (m, 2H, arom.), 7.64 (brs, 2H, arom.), 7.72-7.76 (m, 2H, arom.), 7.83-7.87 (m, 1H, arom.), 7.92, 7.96 (s, 1H, CH).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  193.7, 174.0, 166.9, 165.9 (d,  $J = 294$  Hz), 163.0, 134.4, 133.9, 133.3, 131.5, 131.2, 130.0 (q,  $J = 31$  Hz), 130.0, 125.6 (q,  $J = 270$  Hz), 125.2, 123.7 (d,  $J = 20$  Hz), 121.1, 117.3 (d,  $J = 22$  Hz), 53.1, 33.0. Anal. calcd. for  $\text{C}_{21}\text{H}_{12}\text{F}_4\text{N}_2\text{O}_3\text{S}_2$  (%): C 52.50, H 2.52, N 5.83. Found C 52.30, H 2.30, N 6.00.

*1-(4-Chloro-phenyl)-3-[5-(4-fluoro-benzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-pyrrolidine-2,5-dione (Compound 10)*. Yield: 75% (method A), 53% (method B), mp 206-207 °C [7].  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.24-3.37 (m, 2H, CHCH $_2$ ), 5.97 (dd,  $J = 5.5$  Hz, 9.5 Hz), 6.26 (m, 1H, CHCH $_2$ ), 7.31-7.37 (m, 2H, arom.), 7.42 (t, 2H,  $J = 8.4$  Hz, arom.), 7.60-7.64 (m, 2H, arom.), 7.72-7.76 (m, 2H, arom.), 7.89, 7.98 (s, 1H, CH).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  193.7, 174.1, 172.7, 163.9 (d,  $J = 252$  Hz), 162.9, 134.3, 133.9, 133.8, 133.7, 131.4, 129.8, 129.0, 121.1, 117.3 (d,  $J = 22$  Hz), 53.1, 33.0. Anal. calcd. for  $\text{C}_{20}\text{H}_{12}\text{ClFN}_2\text{O}_3\text{S}_2$  (%): C 53.75, H 2.71, N 6.27; Found C 53.90, H 2.90, N 6.10.

*3-[5-(4-Methoxybenzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-1-(3-trifluoromethylphenyl)-pyrrolidine-2,5-dione (Compound 13)*. Yield: 70% (method A), 62% (method B), mp > 230 °C.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.26-3.31 (m, 2H, CHCH $_2$ ), 3.85 (s, 3H, CH $_3$ ), 5.96, 6.26-2.69 (m, 1H, CHCH $_2$ ), 7.14 (d, 2H,  $J = 8.1$  Hz, arom.), 7.62-7.67 (brs, 4H, arom.), 7.81-7.87 (m, 2H, arom.), 7.85, 7.93 (s, 1H, CH).  $^{13}\text{C}$

NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  184.1, 174.1, 172.8, 162.5, 135.8, 135.2, 133.7, 133.3, 131.6 (q,  $J = 32$  Hz), 131.3, 130.4, 127.2 (q,  $J = 286$  Hz), 126.1, 125.8, 123.8, 117.9, 115.8, 56.2, 53.1, 33.1. Anal. calcd. for  $C_{22}H_{15}F_3N_2O_4S_2$  (%): C 53.65, H 3.07, N 5.69; Found C 53.50, H 2.90, N 5.90.

*3-[4-Oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-1-m-tolyl-pyrrolidine-2,5-dione (Compound 14)*. Yields: 81% (method A), 75% (method B), mp 188-189 °C.  $^1H$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  2.37 (s, 3H,  $CH_3$ ), 3.18-3.26 (m, 2H,  $CHCH_2$ ), 5.93 (dd,  $J = 5.5$  Hz,  $J = 9.5$  Hz), 6.23-6.26 (m, 1H,  $CHCH_2$ ), 7.06-7.09 (m, 2H, arom.), 7.16-7.18 (m, 1H, arom.), 7.26-7.29 (m, 1H, arom.), 7.38-7.47 (m, 5H, arom.), 7.57-7.65 (m, 1H, arom.), 7.73 (d, 2H,  $J = 6.9$  Hz, arom.).  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  193.5, 174.3, 173.0, 166.1, 146.8, 139.1, 135.9, 135.3, 132.6, 130.9, 129.8, 129.5, 129.4, 128.8, 127.7, 124.4, 124.0, 122.6, 53.1, 33.1, 21.3. Anal. calcd. for  $C_{23}H_{18}N_2O_3S_2$  (%): C 63.57, H 4.18, N 6.45; Found C 63.40, H 4.00, N 6.60.

*3-[4-Oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-1-phenyl-pyrrolidine-2,5-dione (Compound 15)*. Yields: 67% (method A), 56% (method B), mp 225-227 °C.  $^1H$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.08-3.35 (m, 2H,  $CH_2$ ), 5.94, 6.25 (m, 1H, CH), 7.06-7.14 (m, 1H,  $CH=CH-CH=$ ), 7.27 (d, 2H,  $J = 7.8$  Hz, arom.), 7.30 (d, 2H,  $J = 7.9$  Hz, arom.), 7.46-7.48 (m, 4H, arom.) 7.49-7.59 (m, 2H, arom.), 7.60-7.70 (m, 2H, arom.).  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$ , ppm: 179.6, 174.4, 173.0, 166.5, 146.9, 135.9, 135.3, 130.9, 129.7, 129.5, 129.2, 128.9, 127.3, 127.2, 124.1, 122.6, 53.1, 33.1. Anal. calcd. for  $C_{22}H_{16}N_2O_3S_2$  (%): C 62.84, H 3.84, N 6.66; Found C 63.00, H 3.70, N 6.80.

*1-(4-Methoxy-phenyl)-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrolidine-2,5-dione (Compound 16)*. Yields: 81% (method A), 75% (method B), mp 211-212 °C.  $^1H$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.05 (dd, 1H,  $J = 17.0$ ,  $J = 5.7$  Hz,  $CHCH_2$ ), 3.14-3.35 (m, 1H,  $CHCH_2$ ), 3.83 (s, 3H,  $OCH_3$ ), 5.88, 6.20 (m, 1H,  $CHCH_2$ ), 7.00-7.22 (m, 3H, arom.), 7.36-7.44 (m, 4H, arom.), 7.56 (d, 2H,  $J = 8.4$  Hz, arom.), 7.64-7.70 (m, 3H, arom.).  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$ : 194.1, 175.1, 173.7, 166.7, 160.2, 147.4, 136.4, 131.4, 130.0, 129.3, 129.0, 128.8, 125.7, 124.5, 123.1, 115.4, 56.4, 53.5, 33.2. Anal. calcd. for  $C_{23}H_{18}N_2O_4S_2$  (%): C 61.32, H 4.03, N 6.22; Found C 61.50, H 3.90, N 6.10.

*1-(4-Ethoxy-phenyl)-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrroli-*

*dine-2,5-dione (Compound 17)*. Yields: 69% (method A), 70% (method B), mp 206-208 °C.  $^1H$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  1.33-1.36 (m, 3H,  $CH_3$ ), 3.15-3.19 (m, 1H,  $CHCH_2$ ), 3.28-3.33 (m, 1H,  $CHCH_2$ ), 4.07 (q, 2H,  $J = 6.7$  Hz,  $CH_2CH_3$ ), 5.90 (dd,  $J = 5.5$  Hz, 9.5 Hz), 6.22 (m, 1H,  $CHCH_2$ ), 7.04-7.09 (m, 2H, arom.), 7.12-7.19 (m, 3H, arom.), 7.42-7.47 (m, 4H, arom.), 7.57-7.66 (m, 1H, arom.), 7.72-7.74 (m, 2H, arom.).  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  193.5, 174.5, 173.1, 166.1, 158.9, 146.8, 135.9, 135.2, 130.8, 129.5, 128.8, 128.5, 128.3, 125.0, 124.0, 115.3, 63.9, 53.0, 33.0, 15.1. Anal. calcd. for  $C_{24}H_{20}N_2O_4S_2$  (%): C 62.05, H 4.34, N 6.03; Found C 62.20, H 4.50, N 5.90.

*3-[4-Oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-1-(2-trifluoromethyl-phenyl)-pyrrolidine-2,5-dione (Compound 18)*. Yields: 82% (method A), 63% (method B), mp 160-162 °C.  $^1H$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.00-3.35 (m, 2H,  $CHCH_2$ ), 5.94, 6.15 (m, 1H,  $CHCH_2$ ), 7.00-7.10 (m, 1H,  $CH=CH-CH=$ ), 7.34-7.46 (m, 3H, arom.), 7.54 (m, 3H, arom.), 7.60-7.72 (m, 5H, arom.).  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  179.4, 174.2, 173.0, 166.6, 146.8, 135.8, 135.4, 130.6, 129.8 (q,  $J = 30$  Hz), 129.4, 129.1, 128.9, 127.4 (q,  $J = 270$  Hz), 127.0, 126.8, 124.0, 122.1, 53.2, 33.1. Anal. calcd. for  $C_{23}H_{15}F_3N_2O_3S_2$  (%): C 56.55, H 3.10, N 5.73; Found: C 56.70, H 3.40, N 5.50.

*3-[4-Oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-1-(3-trifluoromethyl-phenyl)-pyrrolidine-2,5-dione (Compound 19)*. Yields: 67% (method A), 62% (method B), mp 175-177 °C.  $^1H$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.24-3.28 (m, 1H,  $CHCH_2$ ), 3.38-3.43 (m, 1H,  $CHCH_2$ ), 5.92, 6.26 (m, 1H,  $CHCH_2$ ), 7.14-7.19 (m, 1H, arom.), 7.41-7.49 (m, 4H, arom.), 7.59-7.65 (m, 3H, arom.), 7.71-7.75 (m, 2H, arom.), 7.80-7.89 (m, 2H, arom.).  $^{13}C$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$ , ppm: 193.5, 174.0, 172.7, 166.2, 147.0, 135.9, 135.4, 133.3, 131.5, 131.2, 130.9, 129.5, 129.1 (q,  $J = 31$  Hz), 128.8, 124.1 (q,  $J = 272$  Hz), 124.0, 122.5, 53.0, 52.6, 33.0, 32.8. Anal. ( $C_{23}H_{15}F_3N_2O_3S_2$ ) C, H, N. Anal. calcd for  $C_{23}H_{15}F_3N_2O_3S_2$  (%): C 56.55, H 3.10, N 5.73; Found: C 56.40, H 2.90, N 5.90

*General procedure for 5-ylidene-3-(1-aryl-pyrrolidine-2,5-dione)-2,4-thiazolidinediones synthesis (Compounds 7, 8, 11 and 12)*. A mixture of 5-ylidene-2,4-thiazolidinedione (10 mmol) and potassium hydroxide (10 mmol) in ethanol was heated under reflux for 1 h. To the obtained salt (5 mmol) N-arylchlorosuccinimide (5 mmol) in 15 ml of EtOH:DMF mixture, catalytic amounts of potassium iodide and potassium carbonate were added and heated under reflux for 4 h. The reaction mixture

was cooled and poured into water, the formed solid product was filtered off and recrystallized from the appropriate solvent.

*4-{3-[5-(4-Chloro-benzylidene)-2,4-dioxo-thiazolidin-3-yl]-2,5-dioxo-pyrrolidin-1-yl}-benzoic acid ethyl ester (Compound 7)*. Yields: 81%, mp 175-176 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1.40 (t, 3H, *J* = 7.1 Hz, CH<sub>3</sub>CH<sub>2</sub>), 4.37 (q, 2H, *J* = 8.0 Hz, CH<sub>2</sub>), 3.06 (dd, *J* = 18.5 Hz, *J* = 5.6 Hz), 3.20-3.35 (m, 2H, CHCH<sub>2</sub>), 5.95, 6.26 (m, 1H, CH), 7.44 (d, 2H, *J* = 8.1 Hz, arom.), 7.48 (d, 2H, *J* = 8.0 Hz, arom.), 7.50-7.55 (m, 2H, arom.), 7.58-7.64 (m, 2H, arom.), 7.82s, 7.89 (s, 1H, CH). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ 187.5, 175.9, 173.3, 166.0, 156.6, 140.4, 139.7, 137.0, 132.9, 132.6, 131.9, 131.1, 130.6, 127.7, 120.9, 68.1, 62.1, 28.0, 15.1. Anal. calcd. for C<sub>23</sub>H<sub>17</sub>ClN<sub>2</sub>O<sub>6</sub>S (%): C 56.97, H 3.53, N 5.78; Found: C 57.10, H 3.40, N 5.90.

*5-(4-Chloro-benzylidene)-3-(2,5-dioxo-1-p-tolyl-pyrrolidin-3-yl)-thiazolidine-2,4-dione (Compound 8)*. Yields: 90%, mp 186-187 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.40 (s, 3H, CH<sub>3</sub>), 3.05 (dd, *J* = 18.5 Hz, *J* = 5.9 Hz), 3.16-3.35 (m, 2H, CHCH<sub>2</sub>), 5.95, 6.25 (m, 1H, CH), 7.16 (d, 2H, *J* = 7.9 Hz, arom.), 7.20 (d, 2H, *J* = 8.2 Hz, arom.), 7.25-7.31 (m, 1H, arom.), 7.50-7.54 (m, 1H, arom.), 7.58-7.54 (m, 2H, arom.), 7.80, 7.89 (s, 1H, CH). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ: 174.8 173.7, 171.4, 168.7, 139.3, 136.7, 136.4, 134.2, 132.9, 132.6, 130.61, 130.6, 130.5, 127.5, 51.0, 33.9, 21.8. Anal. calcd. for C<sub>21</sub>H<sub>15</sub>ClN<sub>2</sub>O<sub>4</sub>S (%): C 59.09, H 3.54, N 6.56; Found: C 58.90, H 3.40, N 6.70

*3-(2,5-Dioxo-1-phenyl-pyrrolidin-3-yl)-5-(4-fluoro-benzylidene)-thiazolidine-2,4-dione (Compound II)*. Yields: 72%, mp 184-185 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 3.05 (dd, 1H, *J* = 16.7 Hz, *J* = 5.0 Hz), 3.20-3.35 (m, 1H), 5.95, 6.25 (m, 1H), 7.20-7.28 (m, 2H, arom.) 7.50-7.54 (m, 3H, arom.), 7.58-7.64 (m, 3H, arom.), 7.80, 7.89 (s, 1H, CH). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ 77.5, 172.6, 171.8, 167.7, 166.1, 163.1 (d, *J* = 260 Hz), 143.8, 135.6, 133.5, 133.1, 130.4, 128.9, 127.1, 125.6, 117.1 (d, *J* = 22 Hz), 55.2, 33.1. Anal. calcd. For C<sub>20</sub>H<sub>13</sub>FN<sub>2</sub>O<sub>4</sub>S (%): C 60.60, H 3.31, N 7.07; Found: C 60.50, H 3.20, N 7.20.

*4-{3-[5-(4-Fluoro-benzylidene)-2,4-dioxo-thiazolidin-3-yl]-2,5-dioxo-pyrrolidin-1-yl}-benzoic acid ethyl ester (Compound 12)*. Yields: 92%, mp 162-164 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 1,34 (t, 3H, *J* = 6.7 Hz, CH<sub>3</sub>), 3.24-3.29 (m, 2H, CHCH<sub>2</sub>), 4.34-4.36 (m, 2H, CH<sub>2</sub>CH<sub>3</sub>), 5.54-5.58 (m, 1H, CHCH<sub>2</sub>),

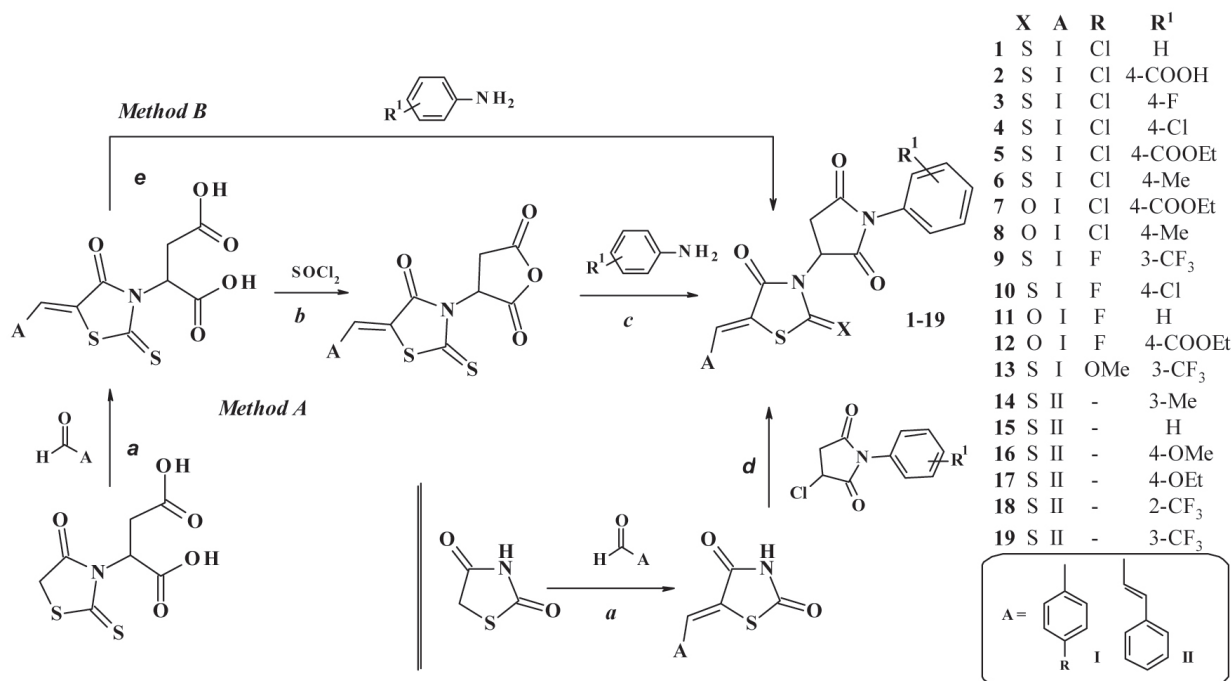
7.41-7.46 (m, 3H, arom.), 7.72-7.74 (brs, 2H, arom.), 7.97-8.09 (m, 2H, arom.), 8.11-8.14 (m, 2H, arom.). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>), δ, ppm: 177.6, 172.8, 171.7, 167.3, 165.5, 162.6 (d, *J* = 272 Hz), 144.1, 135.8, 133.4, 133.3, 130.5, 129.0, 127.3, 125.8, 117.2 (d, *J* = 22 Hz), 61.5, 55.4, 33.1, 14.6. Anal. calcd. for C<sub>23</sub>H<sub>17</sub>FN<sub>2</sub>O<sub>6</sub>S (%): C 58.97, H 3.66, N 5.98; Found: C 59.10, H 3.70, N 5.80.

*Anti-leukemic activity screening*. *In vitro* screening of anticancer activity of the synthesized 19 compounds towards four leukemia cell lines (Dami, HL-60, Jurkat, K562) was measured by the MTT test [48]. Tumor cells were seeded for 24 h in 96-well microtiter plates at a concentration of 2000 cells/well or 10,000 suspension cells/well (100 μl/well). After that cells were incubated for 72 h with additions of the synthesized compounds. 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, St. Louis, Missouri, USA).

*Acute toxicity in vivo*. The experiments were conducted on albino outbred laboratory mice, male (20-25 g). Compounds were dissolved in saline solution (0.9% NaCl) with 1-2 drops of Polysorbate 80 (Tween-80). After dissolution they were administered to mice via an intraperitoneal route in a single dose (time of observation 14 days). The LD<sub>50</sub> was evaluated for 4 or 5 different doses (100, 200, 400, 600, 800 mg/kg) each on 4-6 animals and calculated by the Litchfield-Wilcoxon method [49]. This study was conducted with Danylo Halytsky Lviv National Medical University Ethical Committee Approval № 9 21/12/2018.

## Results

Synthesis of rhodanine derivatives was based on the modification of rhodanine-3-succinic acid and involved several steps: synthesis of 5-ylidene-rhodanine-3-succinic acids which were converted into target imides via the stage of cyclic anhydride formation (Scheme). To avoid this stage, the alternative one-step method for target 5-ylidene-3-(1-arylpyrrolidine-2,5-dione)-rhodanines synthesis was worked out: long term heating of 5-ylidene-rhodanine-3-succinic acid with aromatic amine (1:1) in the acetic acid medium (method B). 2,4-Thiazolidinedione was used as a starting compound for the synthesis of 5-ylidene-3-(1-arylpyrrolidine-2,5-dione)-thiazolidine-2,4-diones. 5-Ylidene-2,4-thiazolidinones were obtained via Knoevenagel condensation and



**Scheme.** Synthesis of target 5-ylidene-4-thiazolidinone-pyrrolidine-2,5-dione hybrids. Reagents and conditions: a) rhodanine-3-succinic acid or 2,4-thiazolidinedione (1 equiv.), aldehyde (1 equiv.), AcONa (1 equiv.), AcOH, reflux 3 h; b) 5-ene-rhodanine-3-succinic acid (1 equiv.), SOCl<sub>2</sub> (3 equiv.), dioxane, reflux, 1 h; c) 5-ene-rhodanine-3-succinic acid anhydride (1 equiv.), amine (1 equiv.), dioxane, reflux reflux, 3 h; d) 1. 5-ene-2,4-thiazolidinedione (1 equiv.), KOH (1 equiv.), EtOH, reflux 1 h; 2. 5-ene-2,4-thiazolidinedione potassium salt (1 equiv.), N-aryl-chlorosuccinimide (1 equiv.), EtOH:DMF, KI, K<sub>2</sub>CO<sub>3</sub> (catalytically amount) reflux 4 h; e) 5-ene-rhodanine-3-succinic acid (1 equiv.), amine (1 equiv.), AcOH, reflux, 12 h

were utilized in the alkylation reaction with N-aryl-2-chlorosuccinimides. The structures and purity of synthesized compounds were confirmed by spectral and analytical data (experimental part).

The tested compounds were evaluated at one dose (10 μM) in the MTT assay (Table). The most sensitive cell line to the tested compounds turned out to be the DAMI line. Four compounds from the rhodanine-based group (**1**, **2**, **9**, and **14**) inhibited its growth by more than 50%. In general, compounds bearing the 2-thioxo-4-thiazolidone core showed higher inhibition rates than their analogs with the thiazolidine-2,4-dione cycle.

The most active compounds (**1**, **2**, **9**, **14**) were evaluated for their approximate LD<sub>50</sub> in albino outbred laboratory mice [49, 55]. Increasing amounts of the compounds (100-1000 mg/kg) were injected intraperitoneally and LD<sub>50</sub> were calculated according to Litchfield and Wilcoxon. The tested compounds had relatively low toxicity and were well tolerated by the mice. The values of LD<sub>50</sub> were: 460±65.0 mg/

kg (Compound **1**), 570±48.0 mg/kg (Compound **2**), 280±30.0 mg/kg (Compound **9**), and 260±31.0 mg/kg (Compound **14**).

## Discussion

The project involved the design of the target compound structures based on our previous study results and the literature data; development of the synthetic protocols and synthesis of thiazolidinone-pyrrolidinedione conjugates; and in vitro screening towards leukemic cell lines and acute toxicity evaluation *in vivo* in mice for the most active compounds.

Target compounds were synthesized based on known approaches [1, 7] that depend on the type of the main core. For the target 5-ylidene-3-(1-aryl-pyrrolidine-2,5-dione)-rhodanines synthesis the simple and efficient protocol starting with 5-ylidenerhodanine-3-dicarboxylic acids was worked out. A series of newly synthesized 5-ene-thiazolidone-3-succinimides were investigated against four leukemia cell lines: Dami, HL-60, Jurkat and K562. K-562 [50] and

Table. The percentage of leukemic cell line growth under the action of the 19 compounds at one dose (10  $\mu$ M) as measured by the MTT test

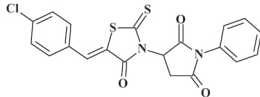
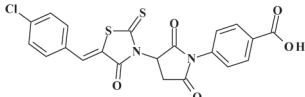
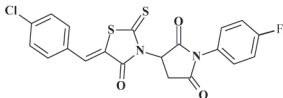
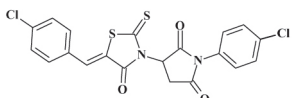
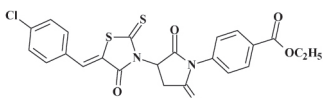
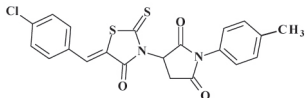
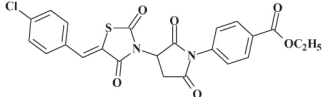
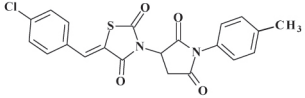
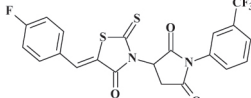
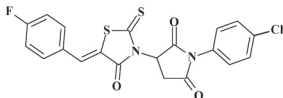
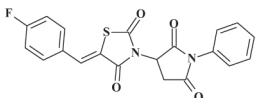
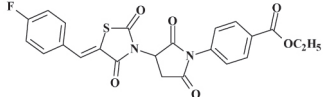
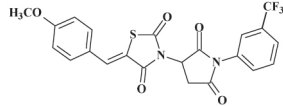
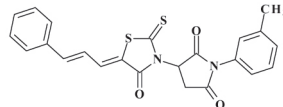
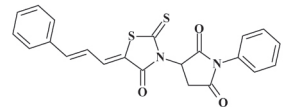
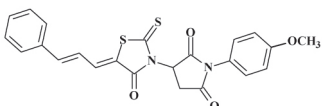
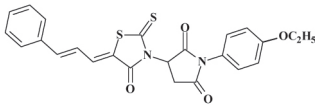
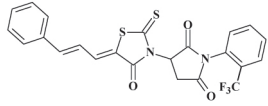
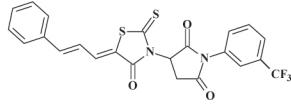
Compound	Cell line growth, %			
	Dami	HL-60	Jurkat	K562
<b>1</b> 	44.75	40.30	71.98	95.12
<b>2</b> 	<b>43.49</b>	58.18	95.41	110.18
<b>3</b> 	75.86	136.69	94.84	89.52
<b>4</b> 	138.55	284.34	112.80	107.19
<b>5</b> 	125.87	68.54	97.068	107.45
<b>6</b> 	101.73	109.97	105.85	113.54
<b>7</b> 	57.93	96.30	102.54	89.37
<b>8</b> 	80.68	120.42	90.31	105.85
<b>9</b> 	<b>35.10</b>	84.57	80.71	98.17
<b>10</b> 	81.77	100.74	103.28	105.26
<b>11</b> 	52.87	122.42	101.89	120.20
<b>12</b> 	88.25	271.54	117.48	110.01

Table. (Continuation)

Compound	Cell line growth, %			
	Dami	HL-60	Jurkat	K562
<b>13</b> 	69.87	166.96	103.53	100.35
<b>14</b> 	<b>48.42</b>	183.52	104.26	115.24
<b>15</b> 	109.25	174.88	98.73	105.39
<b>16</b> 	117.90	167.15	107.44	105.55
<b>17</b> 	87.93	119.30	99.61	100.59
<b>18</b> 	109.57	175.74	100.94	106.05
<b>19</b> 	77.16	198.52	103.22	106.52

Dami [51] cell lines belong to the erythrocytic-megakaryocytic lines – a group that may arise from a common haematopoietic progenitor of both lineages, which are considered close and share some specific transcription factors including GATA-1 and NF-E2 [52]. The HL-60 cell line represents promyelocytic leukemia cells [53] and the Jurkat leukemic T-cell line represents one of the best studied T-cell receptor signaling systems [54].

The best results towards both Dami and HL-60 cell lines were observed for the 3-[5-(4-chlorobenzylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-1-phenylpyrrolidine-2,5-dione (Compound **1**) with no substituent in the arylidene moiety of the pyrrolidine cycle. Although, this lipophilic group in the molecule caused the highest growth inhibition

rates, the carboxylic group in the para position and trifluoromethyl group in the meta position of the phenyl ring are also preferable. Only one hit – compound **1** – showed cytotoxicity against both DAMI and HL-60 leukemic cell lines. The tested compounds have satisfactory toxicity levels (acute toxicity in mice) and meet the requirements for the drug-like molecules.

### Conclusions

A series of 5-ylidene-3-(1-aryl-pyrrolidine-2,5-dione)-2-(thio)oxo-4-thiazolidinones was designed and synthesized. For the synthesis of 3-[5-(arylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-1-arylpyrrolidine-2,5-diones a simple and efficient method that allows the omission of the crucial



step of anhydride formation was developed. Synthesized compounds were tested towards four leukemia cell lines: Dami, HL-60, Jurkat and K562. Hit-compounds – 3-[5-(4-chlorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]-1-phenylpyrrolidine-2,5-dione (Compound **1**) and 3-[5-(4-fluorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]-1-(3-trifluoromethylphenyl)-pyrrolidine-2,5-dione (Compound **9**) showed selective action towards DAMI leukemic cell line that along with low acute toxicity can warrant further in-depth anticancer study of this class of compounds.

*Conflict of interest.* Authors have completed the Unified Conflicts of Interest form at [http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi\\_disclosure.pdf](http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf) and declare no conflict of interest.

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## СИНТЕЗ ТА ПРОТИЛЕЙКЕМІЧНА АКТИВНІСТЬ ПРОЛІДИНОН-ТІАЗОЛІДИНОНИХ ГІБРИДІВ

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Розроблено дизайн структури та синтезовано ряд нових 2-(5-ліден-4-оксо-2-тіоксотіазолідин-3-іл)-сукцинімідів та 5-ліден-3-(1-арилпіролідин-2,5-діон)-тіазолідин-2,4-діонів. Запропоновано ефективний простий метод синтезу роданін-піролідиндіононих гібридів, що дає змогу уникнути стадії утворення ангідриду. Зважаючи на попередні дані щодо протилейкемічної активності споріднених похідних тіазолідінону, біологічна активність цільових сполук досліджувалася на клітинних лініях Dami, HL-

60, Jurkat та K562. Серед досліджених сполук, ідентифіковано 3-[5-(4-хлоробензиліден)-4-оксо-2-тіоксотіазолідин-3-іл]-1-фенілпіролідин-2,5-діон, що володіє доброю і селективною антипроліферативною активністю на лініях Dami та HL-60, а також задовільними токсикологічними параметрами (гостра токсичність *in vivo*).

**Ключові слова:** 4-тіазолідінон, піролідиндіон, протиракова активність, протилейкемічна активність.

## References

1. Kaminsky D, Zimenkovsky B, Lesyk R. Synthesis and in vitro anticancer activity of 2,4-azolidinedione-acetic acids derivatives. *Eur J Med Chem.* 2009; 44(9): 3627-3636.
2. Senkiv J, Finiuk N, Kaminsky D, Havrylyuk D, Wojtyra M, Kril I, Gzella A, Stoika R, Lesyk R. 5-Ene-4-thiazolidinones induce apoptosis in mammalian leukemia cells. *Eur J Med Chem.* 2016; 117: 33-46.
3. Kaminsky D, den Hartog GJM, Wojtyra M, Lelyukh M, Gzella A, Bast A, Lesyk R. Antifibrotic and anticancer action of 5-ene amino/iminothiazolidinones. *Eur J Med Chem.* 2016; 112: 180-195.
4. Kaminsky D, Kryshchyshyn A, Lesyk R. 5-Ene-4-thiazolidinones – an efficient tool in medicinal chemistry. *Eur J Med Chem.* 2017; 140: 542-594.
5. Kaminsky D, Kryshchyshyn A, Lesyk R. Recent developments with rhodanine as a scaffold for drug discovery. *Expert Opin Drug Discov.* 2017; 12(12): 1233-1252.
6. Kaminsky DV, Lesyk RB. Structure-anticancer activity relationships among 4-azolidinone-3-carboxylic acids derivatives. *Biopolym Cell.* 2010; 26(2): 136-145.
7. Kaminsky DV, Roman OM, Atamanyuk DV, Lesyk RB. 5-Ylidene-2-thioxo-4-thiazolidinone-3-succinic acids and their derivatives: synthesis, anticancer activity, QSAR-analysis. *J Org Pharm Chem.* 2006; 4(1(13)): 41-48. (In Ukrainian).
8. Zheng CJ, Song MX, Sun L, Wu Y, Hong L, Piao HR. Synthesis and biological evaluation of 5-aryloxypyrazole derivatives bearing a rhodanine-3-aromatic acid as potential antimicrobial agents. *Bioorg Med Chem Lett.* 2012; 22(23): 7024-7028.

9. Liu JC, Zheng CJ, Wang MX, Li YR, Ma LX, Hou S, Piao HR. Synthesis and evaluation of the antimicrobial activities of 3-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-2-thioxothiazolidin-4-one derivatives. *Eur J Med Chem.* 2014; 74: 405-410.
10. Jin X, Zheng CJ, Song MX, Wu Y, Sun LP, Li YJ, Yu LJ, Piao HR. Synthesis and antimicrobial evaluation of L-phenylalanine-derived C5-substituted rhodanine and chalcone derivatives containing thiobarbituric acid or 2-thioxo-4-thiazolidinone. *Eur J Med Chem.* 2012; 56: 203-209.
11. Liu XF, Zheng CJ, Sun L, Liu XK, Piao HR. Synthesis of new chalcone derivatives bearing 2,4-thiazolidinedione and benzoic acid moieties as potential anti-bacterial agents. *Eur J Med Chem.* 2011; 46(8): 3469-3473.
12. Tomašić T, Kovač A, Simčić M, Blanot D, Grdadolnik SG, Gobec S, Kikelj D, Mašič LP. Novel 2-thioxothiazolidin-4-one inhibitors of bacterial MurD ligase targeting D-Glu- and diphosphate-binding sites. *Eur J Med Chem.* 2011; 46(9): 3964-3975.
13. Orchard MG, Neuss JC, Galley CMS, Carr A, Porter DW, Smith P, Scopes DIC, Haydon D, Vousden K, Stubberfield CR, Young K, Page M. Rhodanine-3-acetic acid derivatives as inhibitors of fungal protein mannosyl Transferase 1 (PMT1). *Bioorg Med Chem Lett.* 2004; 14(15): 3975-3978.
14. Pudhom K, Kasai K, Terauchi H, Inoue H, Kaiser M, Brun R, Ihara M, Takasu K. Synthesis of three classes of rhodacyanine dyes and evaluation of their in vitro and in vivo antimalarial activity. *Bioorg Med Chem.* 2006; 14(24): 8550-8563.
15. Smith TK, Young BL, Denton H, Hughes DL, Wagner GK. First small molecular inhibitors of *T. brucei* dolicholphosphate mannose synthase (DPMS), a validated drug target in African sleeping sickness. *Bioorg Med Chem Lett.* 2009; 19(6): 1749-1752.
16. Choi J, Ko Y, Lee HS, Park YS, Yang Y, Yoon S. Identification of (b-carboxyethyl)-rhodanine derivatives exhibiting peroxisome proliferator-activated receptor  $\gamma$  activity. *Eur J Med Chem.* 2010; 45(1): 193-202.
17. Maccari R, Paoli P, Ottanà R, Jacomelli M, Ciurleo R, Manao G, Steindl T, Langer T, Vigorita MG, Camici G. 5-Arylidene-2,4-thiazolidinediones as inhibitors of protein tyrosine phosphatases. *Bioorg Med Chem.* 2007; 15(15): 5137-5149.
18. Kaminsky D, Bednarczyk-Cwynar B, Vasylenko O, Kazakova O, Zimenkovsky B, Zaprutko L, Lesyk R. Synthesis of new potential anticancer agents based on 4-thiazolidinone and oleanane scaffolds. *Med Chem Res.* 2012; 21(11): 3568-3580.
19. Suresh N, Nagesh HN, Sekhar KVG, Kumar A, Shirazi AN, Parang K. Synthesis of novel ciprofloxacin analogues and evaluation of their anti-proliferative effect on human cancer cell lines. *Bioorg Med Chem Lett.* 2013; 23(23): 6292-6295.
20. Sun CL, Christensen JG, McMahon G. Chapter 1. In: Li R, Stafford JA, eds. Kinase Inhibitor Drugs. Hoboken, New Jersey.: John Wiley Sons, Inc, 2009.
21. Szychowski KA, Leja ML, Kaminsky DV, Binduga UE, Pinyazhko OR, Lesyk RB, Gmiński J. Study of novel anticancer 4-thiazolidinone derivatives. *Chem Biol Interact.* 2017; 262: 46-56.
22. Salamone S, Colin C, Grillier-Vuissoz I, Kuntz S, Mazerbourg S, Flament S, Martin H, Richert L, Chapleur Y, Boisbrun M. Synthesis of new troglitazone derivatives: anti-proliferative activity in breast cancer cell lines and preliminary toxicological study. *Eur J Med Chem.* 2012; 51: 206-215.
23. Jain VS, Vora DK, Ramaa CS. Thiazolidine-2,4-diones: progress towards multifarious applications. *Bioorg Med Chem.* 2013; 21(7): 1599-1620.
24. Szychowski KA, Leja ML, Kaminsky DV, Kryshchshyn AP, Binduga UE, Pinyazhko OR, Lesyk RB, Tobiasz J, Gmiński J. Anticancer properties of 4-thiazolidinone derivatives depend on peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ). *Eur J Med Chem.* 2017; 141: 162-168.
25. Chandrappa S, Kavitha CV, Shahabuddin MS, Vinaya K, Kumar CSA, Ranganatha SR, Raghavan SC, Rangappa KS. Synthesis of 2-(5-((5-(4-chlorophenyl)furan-2-yl)methylene)-4-oxo-2-thioxo-thiazolidin-3-yl)acetic acid derivatives and evaluation of their cytotoxicity and induction of apoptosis in human leukemia cells. *Biorg Med Chem.* 2009; 17(6): 2576-2584.
26. Michalic L, Desverge B, Wahli W. Peroxisome-proliferator-activated receptors and cancers:

- complexes stories. *Nat Rev Cancer*. 2004; 4(1): 61-70.
27. Kubota T, Koshizuka K, Williamson EA, Asou H, Said JW, Holden S, Miyoshi I, Koeffler P. Ligand for peroxisome proliferator-activated receptor  $\gamma$  (Troglitazone) has potent antitumor effect against human prostate cancer both in vitro and in vivo. *Cancer Res*. 1998; 58(15): 3344-3352.
28. Ammazalorso A, De Filippis B, Giampietro L, Amoroso R. Blocking the peroxisome proliferator-activated receptor (PPAR): an overview. *Chem Med Chem*. 2013; 8(10): 1609-1616.
29. Panigrahy D, Huang S, Kieran MW, Kaipainen A. PPAR $\gamma$  as a therapeutic target for tumor angiogenesis and metastasis. *Cancer Biol Ther*. 2005; 4(7): 687-693.
30. Cutshall NS, O'Day C, Prezhdo M. Rhodanine derivatives as inhibitors of JSP-1. *Bioorg Med Chem Lett*. 2005; 15(14): 3374-3379.
31. Fu H, Hou X, Wang L, Dun Y, Yang X, Fang H. Design, synthesis and biological evaluation of 3-aryl-rhodanine benzoic acids as anti-apoptotic protein Bcl-2 Inhibitors. *Bioorg Med Chem Lett*. 2015; 25(22): 5265-5269.
32. Liu W, Bulgaru A, Haigentz M, Stein CA, Perez-Soler R, Mani S. The Bcl2-family of protein ligands as cancer drugs: the next generation of therapeutics. *Curr Med Chem. Anticancer Agents*. 2003; 3(3): 217-223.
33. Lugovskoy AA, Degterev AI, Fahmy AF, Zhou P, Gross JD, Yuan J, Wagner GA. A novel approach for characterizing protein ligand complexes: molecular basis for specificity of small-molecule Bcl-2 inhibitors. *J Am Chem Soc*. 2002; 124(7): 1234-1240.
34. Xing C, Wang L, Tang X, Sham YY. Development of selective inhibitors for anti-apoptotic Bcl-2 proteins from BHI-1. *Bioorg Med Chem*. 2007; 15(5): 2167-2176.
35. Shiau CW, Yang CC, Kulp SK, Chen KF, Chen CS, Huang JW, Chen CS. Thiazolidinediones mediate apoptosis in prostate cancer cells in part through inhibition of Bcl-xL/Bcl-2 functions independently of PPAR $\gamma$ . *Cancer Res*. 2005; 65(4): 1561-1569.
36. Tomašić T, Peterlin Mašić LP. Rhodanine as a scaffold in drug discovery: a critical review of its biological activities and mechanisms of target modulation. *Expert Opin Drug Discov*. 2012; 7(7): 549-560.
37. Mendgen T, Steuer C, Klein CD. Privileged scaffolds or promiscuous binders: a comparative study on rhodanines and related heterocycles in medicinal chemistry. *J Med Chem*. 2012; 55(2): 743-753.
38. Baell JB, Holloway GA. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. *J Med Chem*. 2010; 53(7): 2719-2740.
39. Pinson JA, Schmidt-Kittler O, Frazzetto M, Zheng Z, Jennings IG, Kinzler KW, Vogelstein B, Chalmers DK, Thompson E. Synthesis and pharmacological evaluation of 4-iminothiazolidinones for inhibition of PI3 kinase. *Aust J Chem*. 2012; 65(10): 1396-1404.
40. Zhou H, Wu S, Zhai S, Liu A, Sun Y, Li R, Zhang Y, Ekins S, Swaan PW, Fang B, Zhang B, Yan B. Design, synthesis, cytoselective toxicity, structure activity relationships, and pharmacophore of thiazolidinone derivatives targeting drug-resistant lung cancer cells. *J Med Chem*. 2008; 51(5): 1242-1251.
41. Smelcerovic Z, Veljkovic A, Kocic G, Yancheva D, Petronijevic Z, Anderluh M, Smelcerovic A. Xanthine oxidase inhibitory properties and anti-inflammatory activity of 2-amino-5-alkylidene-thiazol-4-ones. *Chem Biol Interact*. 2015; 229: 73-81.
42. Morphy R, Rankovic Z. Designed multiple ligands. An emerging drug discovery paradigm. *J Med Chem*. 2005; 48(21): 6523-6543.
43. Ge X, Wakim B, Sem DS. Chemical proteomics-based drug design: target and antitarget fishing with a catechol – rhodanine privileged scaffold for NAD(P)(H) binding proteins. *J Med Chem*. 2008; 51(15): 4571-4580.
44. Kryshchshyn AP, Atamanyuk DV, Kaminsky DV, Grellier Ph, Lesyk RB. Investigation of anticancer and anti-parasitic activity of thiopyrano[2,3-d]thiazoles bearing norbornane moiety. *Biopolym Cell*. 2017; 33(3): 183-205.
45. Kryshchshyn A, Roman O, Lozynskyi A, Lesyk R. Thiopyrano[2,3-d]thiazoles as new efficient scaffolds in medicinal chemistry. *Sci Pharm*. 2018; 86(2): 26.
46. Kavitha CV, Chandrappa S, Narasimhamurthy KH, Rangappa KS. Synthesis and evaluation of 5-((5-(4-methoxyphenyl)furan-2-yl) methylene)thiazolidine-2,4-diones as a new

- class of cytotoxic agents for leukemia treatment. *Asian J Biochem Pharm Res.* 2014; 4: 309-323.
47. Kaminsky D, Subtel'na I, Zimenkovsky B, Karpenko O, Gzella A, Lesyk R. Synthesis and evaluation of anticancer activity of 5-ylidene-4-aminothiazol-2(5H)-one derivatives. *Med Chem.* 2015; 11(6): 517-530.
  48. Liu X, Zu YG, Fu YJ, Yao LP, Gu CB, Wang W, Efferth T. Antimicrobial activity and cytotoxicity towards cancer cells of *Melaleuca alternifolia* (teatree) oil. *Eur Food Res Technol.* 2009; 229(2): 247-253.
  49. Litchfield JT, Wilcoxon F. A simplified method of evaluating dose-effect experiments. *J Pharmacol Exp Ther.* 1949; 96(2): 99-113.
  50. Andersson LC, Nilsson K, Gahmberg CG. K562 – a human erythroleukemic cell line. *Int J Cancer.* 1979; 23(2): 143-147.
  51. Greenberg SM, Rosenthal DS, Greeley TA, Tantravahi R, Handin RI. Characterization of a new megakaryocytic cell line: the Dami cell. *Blood.* 1988; 72(6): 1968-1977.
  52. Saito H. 3 Megakaryocytic cell lines. *Baillieres Clin Haematol.* 1997; 10(1): 47-63.
  53. Collins SJ. The HL-60 promyelocytic leukemia cell line: proliferation, differentiation, and cellular oncogene expression. *Blood.* 1987; 70(5): 1233-1244.
  54. Abraham RT, Weiss A. Jurkat T cells and development of the T-cell receptor signalling paradigm. *Nat Rev Immunol.* 2004; 4(4): 301-308.
  55. Smith WG. 1 Pharmacological Screening Tests. Eds. Ellis GP, West GB. *Progress in Medicinal Chemistry.* 1961; 1: 1-33.