# DRUG DEVELOPMENT

UDC 615.012.1:547.79 doi: https://doi.org/10.15407/ubj92.02.108

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

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**Received:** 22 December 2019; **Accepted:** 27 March 2020

*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).*

*K e y w o r d s: 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-thiazolidonones 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 sourses and used without purification. 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 ±0.4% of the theoretical values. The $^{1}$H NMR spectra were recorded on Varian Gemini 400 MHz (Palo Alto, California, USA) 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.

***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. $^{1}$H NMR (400 MHz, DMSO-$d_6$): δ 3.21-3.29 (m, 2H, CH,$CH_2$), 5.98, 6.27 (m, 1H, $CHCH_2$), 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, CH=). $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 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 $C_{20}H_{13}ClN_2O_3S_2$ (%): 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. $^{1}$H NMR (400 MHz, DMSO-$d_6$): δ 3.24-3.28 (m, 2H, $CHCH_2$), 5.99 (dd, $J$= 6.6 Hz, 9.5 Hz), 6.26-6.29 (m, 1H, $CHCH_2$), 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). $^{13}$C NMR (100 MHz, DMSO-$d_6$), δ, 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 $C_{21}H_{13}ClN_2O_5S_2$ (%): 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. $^1$H NMR (400 MHz, DMSO-$d_6$): δ 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). $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 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 $C_{20}H_{12}ClFN_2O_3S_2$ (%): 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. $^1$H NMR (400 MHz, DMSO-$d_6$): δ 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). $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 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 $C_{20}H_{12}Cl_2N_2O_3S_2$ (%): 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-yrrolidin-1-yl}-benzoic acid ethyl ester (Compound **5**).* Yields: 72% (method A), 51% (method B), mp 239-240 °C. $^1$H NMR (400 MHz, DMSO-$d_6$): δ 1,33 (t, 3H, *J* = 7.0 Hz, $CH_3$), 3.23-3.31 (m, 2H, CHCH2), 4.28-4.30 (m, 2H, $CH_2CH_3$), 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). $^{13}$C NMR (100 MHz, DMSO-$d_6$), δ, 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 $C_{23}H_{17}ClN_2O_5S_2$ (%): 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. $^1$H NMR (400 MHz, DMSO-$d_6$): δ 2.40 (s, 3H, $CH_3$), 3,05 (dd, *J* = 18.5 Hz, *J* = 5.9 Hz), 3.16-3.35 (m, 2H, CHCH2), 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). $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 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 $C_{21}H_{15}ClN_2O_3S_2$ (%): 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]. $^1$H NMR (400 MHz, DMSO-$d_6$): δ 3.28-3.31 (m, 1H, CHCH2), 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). $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 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 $C_{21}H_{12}F_4N_2O_3S_2$ (%): 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]. $^1$H NMR (400 MHz, DMSO-$d_6$): δ 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). $^{13}$C NMR (100 MHz, DMSO-$d_6$): δ 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 $C_{20}H_{12}ClFN_2O_3S_2$ (%): 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. $^1$H NMR (400 MHz, DMSO-$d_6$): δ 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). $^{13}$C

NMR (100 MHz, DMSO-$d_6$): δ 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. $^1$H NMR (400 MHz, DMSO-$d_6$): δ 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-$d_6$) δ 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. $^1$H NMR (400 MHz, DMSO-$d_6$): δ 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-$d_6$), δ, 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. $^1$H NMR (400 MHz, DMSO-$d_6$): δ 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-$d_6$): δ: 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]-pyrrolidine-2,5-dione (Compound **17**).* Yields: 69% (method A), 70% (method B), mp 206-208 °C. $^1$H NMR (400 MHz, DMSO-$d_6$): δ 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, CHCH2), 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-$d_6$): δ 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. $^1$H NMR (400 MHz, DMSO-$d_6$): δ 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-d6): δ 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-$d_6$): δ 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-$d_6$), δ, 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 refluxed 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. $^1$H NMR (400 MHz, DMSO-$d_6$): δ 1.40 (t, 3H, *J* = 7.1 Hz, $CH_3CH_2$), 4.37 (q, 2H, *J* = 8.0 Hz, $CH_2$), 3.06 (dd, J = 18.5 Hz, J = 5.6 Hz), 3.20-3.35 (m, 2H, $CHCH_2$), 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). $^{13}$C NMR (100 MHz, DMSO-$d_6$): 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 $C_{23}H_{17}ClN_2O_6S$ (%): 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. $^1$H NMR (400 MHz, DMSO-$d_6$): δ 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, 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). $^{13}$C NMR (100 MHz, DMSO-$d_6$): 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 $C_{21}H_{15}ClN_2O_4S$ (%): 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 **11**).* Yields: 72%, mp 184-185 °C. $^1$H NMR (400 MHz, DMSO-$d_6$): 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). $^{13}$C NMR (100 MHz, DMSO-$d_6$): 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 $C_{20}H_{13}FN_2O_4S$ (%): C 60.60, H 3.31, N 7.07; Found: C 60.50, H 3.20, N 7.20.

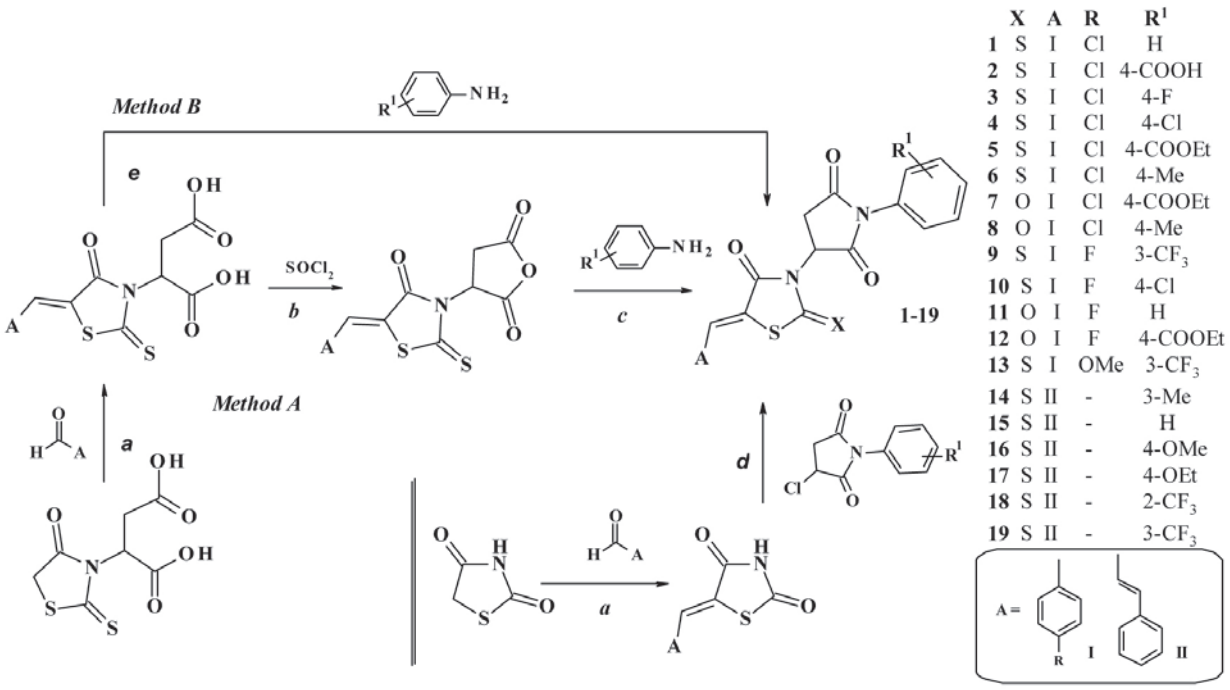
*4-{3-[5-(4-Fluoro-benzylidene)-2,4-dioxo-thiazolidin-3-yl]-2,5-dioxo-pyrrolidin-1-yl}-benzoic acid ethyl ester (Compound **12**).* Yields: 92%, mp 162-164 °C. $^1$H NMR (400 MHz, DMSO-$d_6$): δ 1,34 (t, 3H, *J* = 6.7 Hz, $CH_3$), 3.24-3.29 (m, 2H, $CHCH_2$), 4.34-4.36 (m, 2H, $CH_2CH_3$), 5.54-5.58 (m, 1H, $CHCH_2$), 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.). 13C NMR (100 MHz, DMSO-$d_6$), 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 $C_{23}H_{17}FN_2O_6S$ (%): 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 l-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 $LD_{50}$ was evaluated for 4 or 5 different doses (100, 200, 400, 600, 800 mg/kg) each on 4-6 animals and calculated by the Litchfielde-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




| | X | A | R | R¹ |
|---|---|---|---|---|
| 1 | S | I | Cl | H |
| 2 | S | I | Cl | 4-COOH |
| 3 | S | I | Cl | 4-F |
| 4 | S | I | Cl | 4-Cl |
| 5 | S | I | Cl | 4-COOEt |
| 6 | S | I | Cl | 4-Me |
| 7 | O | I | Cl | 4-COOEt |
| 8 | O | I | Cl | 4-Me |
| 9 | S | I | F | 3-$CF_3$ |
| 10 | S | I | F | 4-Cl |
| 11 | O | I | F | H |
| 12 | O | I | F | 4-COOEt |
| 13 | S | I | OMe | 3-$CF_3$ |
| 14 | S | II | - | 3-Me |
| 15 | S | II | - | H |
| 16 | S | II | - | 4-OMe |
| 17 | S | II | - | 4-OEt |
| 18 | S | II | - | 2-$CF_3$ |
| 19 | S | II | - | 3-$CF_3$ |

*Scheme. Synthesis of target 5-ylidene-4-thiazolidinone-pyrrolidine-2,5-dione hybrids. Reagents and conditions: a) rhodanine-3-succinic acid or 2.4-thiazolidinesione (1 equiv.), aldehyde (1 equiv.), AcONa (1 equiv.), AcOH, reflux 3 h; b) 5-ene-rhodanine-3-succinic acid (1 equiv.), SOCl2 (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-thiazolidinerdione (1 equiv.), KOH (1 equiv.), EtOH, reflux 1 h; 2. 5-ene-2,4-thiazolidinerdione potassium salt (1 equiv.), N-aryl-chlorosuccinimide (1 equiv.), EtOH:DMF, KI, $K_2CO_3$ (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.

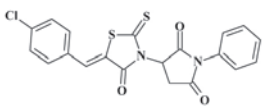
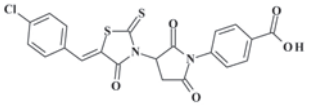
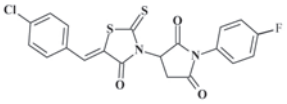
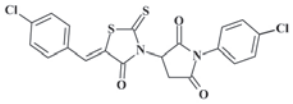
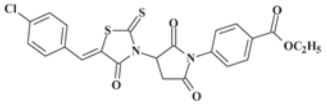
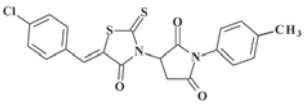
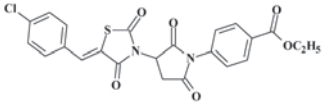
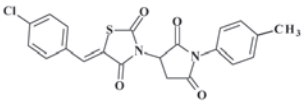
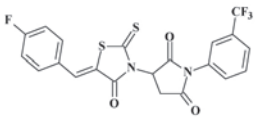
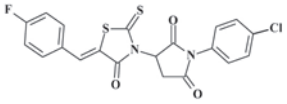
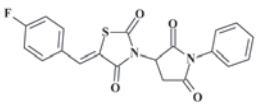
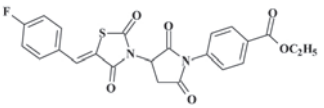
The most active compounds (**1**, **2**, **9**, **14**) were evaluated for their approximate $LD_{50}$ in albino outbred laboratory mice [49, 55]. Increasing amounts of the compounds (100-1000 mg/kg) were injected intraperitoneally and $LD_{50}$ were calculated according to Litchfield and Wilcoxon. The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

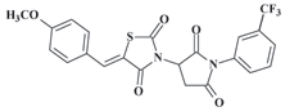
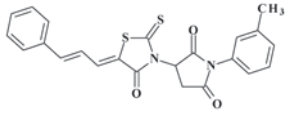
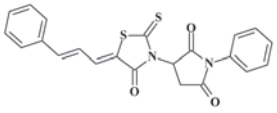
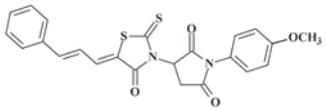
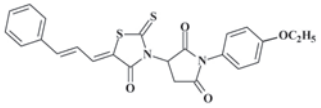
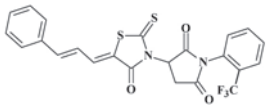
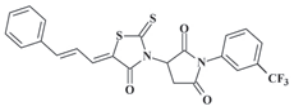
The project involved the design of the target compound structures based on our previous study results and the literature data; development of the synthetic protocols and synthesis of thiazolidinone-pyrrolidinedione conjugates; and in vitro screening towards leukemic cell lines and acute toxicity evaluation *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 μM) as measured by the MTT test*

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

*T a b l e . (Continuation)*

| Compound | | Cell line growth, % | | | |
|---|---|---|---|---|---|
| | | Dami | HL-60 | Jurkat | K562 |
| **13** |  | 69.87 | 166.96 | 103.53 | 100.35 |
| **14** |  | **48.42** | 183.52 | 104.26 | 115.24 |
| **15** |  | 109.25 | 174.88 | 98.73 | 105.39 |
| **16** |  | 117.90 | 167.15 | 107.44 | 105.55 |
| **17** |  | 87.93 | 119.30 | 99.61 | 100.59 |
| **18** |  | 109.57 | 175.74 | 100.94 | 106.05 |
| **19** |  | 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-phenyl-pyrrolidine-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 metha 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-(thi)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 and declare no conflict of interest.

*Acknowledgements.* The authors thank the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) and Dr. Sandor Vari (president of the RECOOP HST Association) for their support. This work was partially supported by the Ministry of Education and Science of Ukraine (№ M/92-2018).

## СИНТЕЗ ТА ПРОТИЛЕЙКЕМІЧНА АКТИВНІСТЬ ПІРОЛІДИНДІОН-ТІАЗОЛІДИНОНОВИХ ГІБРИДІВ

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Розроблено дизайн структури та синтезовано ряд нових 2-(5-іліден-4-оксо-2-тіоксотіазоілдин-3-іл)-сукцинімідів та 5-іліден-3-(1-арилпіролідин-2,5-діон)-тіазоілдин-2,4-діонів. Запропоновано ефективний простий метод синтезу роданін-піролідиндіонових гібридів, що дає змогу уникнути стадії утворення ангідриду. Зважаючи на попередні дані щодо протилейкемічної активності споріднених похідних тіазолідинону, 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-тіазолідинон, піролідиндіон, протиракова активність, протилейкемічна активність.

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