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Medicinal Chemistry, **2015**, *11*, 517-530

Synthesis and Evaluation of Anticancer Activity of 5-Ylidene-4-Aminothiazol-2(5*H*)-one Derivatives

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Abstract: The synthesis and antitumor activity screening of 4-aminothiazol-2(5H)-one derivatives were performed. The absence of possible 4-amino-imino tautomerism of thiazolidinones-2 has been confirmed based on the study of the molecule structures. The existence of the alone amino-form was confirmed. An anticancer activity screening was performed within the Developmental Therapeutics Program (National Cancer Institute/NIH, USA). Tested compounds possess low to moderate anticancer activity (average values - 60 cancer cell lines assay) with significant selective action on certain cancer cell lines (CCRF-CEM and RPMI-8226/leukemia, U251/CNS cancer, RFX 393/renal cancer, OVCAR/ovarian cancer etc.). The advantage of 5-ylidene-4-R-amino derivatives in comparison with compounds with free amino group was shown. Some structure-activity findings, the comparison of target compounds with isomeric 5-ylidene-2-imino(amino)thiazol-4(5H)-ones, as well as COMPARE analysis were described. Among the tested compounds (Z)-5-(furan-2-ylmethylidene)-4-(4-chlorophenylamino)thiazol-2(5H)-one (**IIIk**) and (Z)-5-(4-diethylaminophenylmethylidene)-4-(4-hydroxy-5-isopropyl-2-methylphenylamino)thiazol-2(5H)-one (**IIIp**) possessed the highest levels of activity.

Keywords: Anticancer activity, 4-(Imino)amino-2-thiazolidinone, synthesis, X-ray study.

INTRODUCTION

The exploitation of thiazolidinone scaffolds in the drug discovery remains in demand in the last decade [1-4]. Moreover they belong to the privileged structures in modern medicinal chemistry [5, 6]. The diversity of chemical modifications, isosteric atoms exchanges etc. makes them the attractive scaffolds in structure-based design, as well as privileged substructure-based diversity oriented synthesis [7]. Advances in the identification of lead-compounds and drug-candidates are mainly associated with 2,4-thiazolidinone and 2-thioxo-4-thiazolidinone (rhodanine) cores and summarized at a number of reviews and critical papers [1, 6, 8] (Fig. **1**). In contrast, data regarding isorhodanine (4-thioxo-2-thiazolidinone) [9-11] and especially 4-amino(imino)thiazolidinones are rather scrimpy and limited. Mentioned heterocycles can be considered as bioisosters of 2,4-thiazolidinedione and rhodanine, as well as means for diversification of privileged structures. Despite the foregoing, since prof. I. Komaritsa's first communications in the 60's of the last century [12, 13] information regarding 4-amino(imino)thiazolidinones is undeservedly scarcely reported [14].

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The 4-amino(imino)thiazolidinone-based fused heterocyclic systems [15, 16] are also scarcely described. Notably, examples of them were synthesized in our laboratory for the first time [17]. Concerning thiazolidinone derivatives – they greatly expand the arsenal of heterocyclic-based structures [18, 19]. We assume that the patterns of biological activity of 2,4-thiazolidinedione and rhodanine derivatives, as well as discovered findings and relationships in this area, can also be expected for 4-amino(imino)thiazolidinones derivatives. It should be highlighted, that the 2-amino(imino)thiazolidinone derivatives, which are positional isomers of target 4-amino(imino) derivatives, possess significant pharmacological potential, particularly as anticancer agents [20-23].

Considering the variety of thiazolidinones, the 5-ylidene subtype is of a special interest as source of lead-compounds and drug-candidates, following the thesis about decisive role of the presence and nature of C5 substituent in the thiazolidinone core for realization of the biological effects [3, 6, 24-29]. For instance, the 5-arylidene-4-imino-2-thiazolidinone derivatives have been shown to act as inhibitors of glycogen synthase kinase-3, which emerges as a key therapeutic target for type-2 diabetes, Alzheimer's disease, cancer, and chronic inflammation [30]. Screening of more than 13000 compounds for antimalarial agents search using the agglomerative structural clustering technique allowed identifying 47 starting points for lead optimization including 4-amino(imino)thiazolidinone derivatives [31]. Also 5-ylidene-

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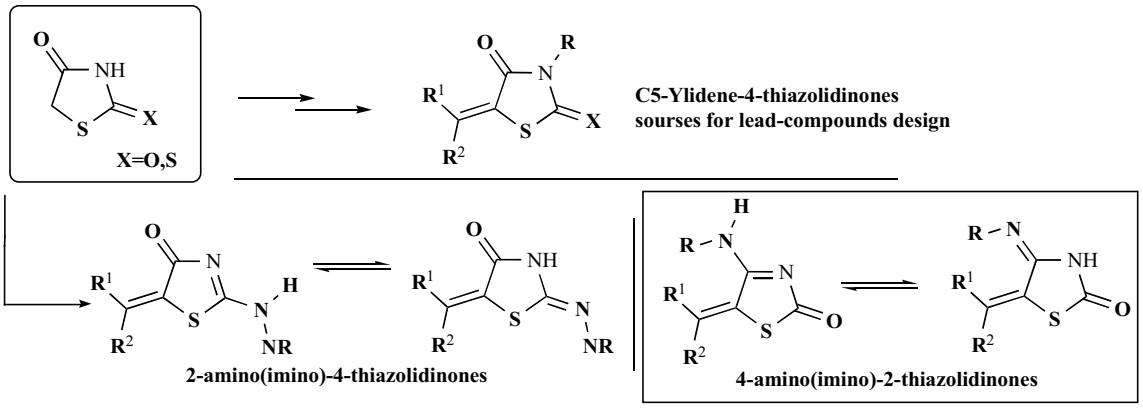
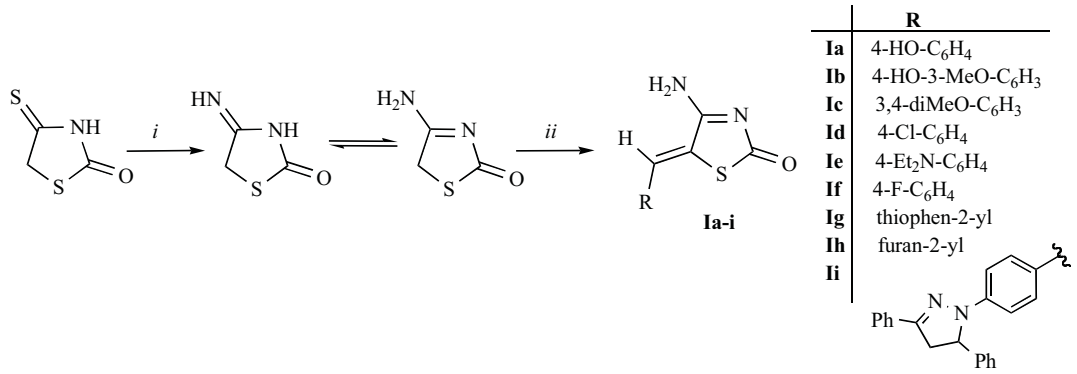


Fig. (1). Diversification of privileged substructure-based oriented synthesis.



Scheme 1. Synthesis of 5-ylidene-4-aminothiazol-2(5*H*)-ones. Reagents and conditions: (*i*) – 4-thioxo-2-thiazolidinone (1.0 equiv), NH_3 (1.0 equiv) 25% solution, 80°C, 15 min; (*ii*) – 4-aminothiazol-2(5*H*)-ones (1 equiv), RCHO (1.0 equiv), AcONa (1.0 equiv), AcOH, reflux, 3 h.

4-aminothiazolidinones can be considered as an estrogen-related receptor modulators and potentially useful as agents for the prevention or treatment of estrogen-related receptor associated diseases such as malignant tumor (*i.e.* breast cancer, malignant lymphoma, multiple myeloma, prostate cancer, colorectal cancer, lung cancer, ovarian cancer, endometrial carcinoma) [32].

Thus, following our studies in the new anticancer agents search the aim of present paper was the synthesis and evaluation of anticancer activity of 4-aminothiazol-2(5*H*)-ones derivatives.

RESULTS AND DISCUSSION

Chemistry

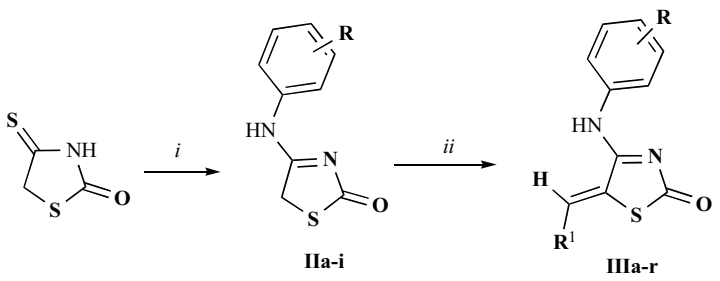
Based on the above mentioned arguments the first step was the synthesis of simple 5-ylidene-4-aminothiazol-2(5*H*)-ones (**I**) (Scheme **1**). The starting 4-aminothiazol-2(5*H*)-one (4-imino-2-thiazolidinone) was obtained *via* reaction of isorhodanine with ammonia solution, as described previously at our department [12].

5-Ylidene derivatives (**Ia-i**) were obtained with high yields *via* Knoevenagel condensation based on the CH-acidic sites analogous to 2,4-thiazolidinedione and rhodanine cores. The reaction was performed in the acetic acid medium in the presence of sodium acetate. The mentioned protocol is much simpler and more efficient compared to the offered one [33].

In the ^{1}H NMR spectra of target 5-ylidene-4-aminothiazol-2(5*H*)-ones two broad singlets in the weak magnetic field were observed. It is consistent with the signals of free amino group. But, similar set could be related to 4-imino form (2 singlets for exo- and endocyclic NH-groups) [34]. The doubling of signals is not observed in contrast to the isomeric 2-amino(imino)-4-thiazolidinones [20], thus the presence of amino-imino prototropic tautomerism for 5-arylidene-4-aminothiazol-2(5*H*)-ones cannot be confirmed. The main argument for representation of compounds in amino form is the X-ray data of related compound (see below). The location of CH= group signal (singlet) at 7.70-8.50 ppm confirms the formation of compounds with *cis* position of arylidene residue (*Z*-configuration), similar to other derivatives of 4-azolidinones [20, 24, 27, 28].

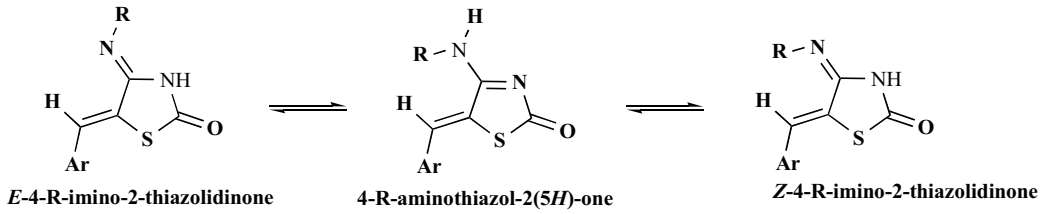
The next stage was the synthesis of 5-ylidene-4-R-aminothiazol-2(5*H*)-ones (Scheme **2**). The approach to the target compounds synthesis (**IIIa-p**) has been developed in our laboratory [12, 13, 35] and widely used in synthetic protocols and included the phase of 4-amino derivatives (**II**) obtaining with the following modifications at position C5.

Such approach is more efficient in comparison with others, including those comprising the step of 5-ylidene-isorhodanines obtaining [14, 33, 36, 37], also in the case of secondary amines utilization [21, 22]. Structurally related compounds have been previously reported as 4-amino- [33] or often as 4-imino derivatives [14, 15]. In both cases the structures of compounds were confirmed by IR-, NMR-spectroscopy and mass-spectrometry. The spectral data didn't contradict with the presented structures. As mentioned above the isomeric 2-arylaminothiazol-4(5*H*)-ones (without



	R	R^1
IIIa	H	4-HO-C_6H_4
IIIb	H	thiophen-2-yl
IIIc	H	furan-2-yl
IIId	4-HO	4-Cl-C_6H_4
IIIe	4-HO	4-F-C_6H_4
IIIf	4-HO	4-HO-C_6H_4
IIIg	4-HO	thiophen-2-yl
IIIh	4-EtO	PhCHCH
IIIi	4-F	4-Cl-C_6H_4
IIIj	4-F	thiophen-2-yl
IIIk	4-Cl	furan-2-yl
IIIl	4-Cl	thiophen-2-yl
IIIm	4-Me	4-F-C_6H_4
IIIn	3-Me	furan-2-yl
IIIo	2-HO	4-Cl-C_6H_4
IIIp	2-Me-4-OH-5-*i*-Pr	4-Et_2N-C_6H_4
IIIq	2-Me-4-OH-5-i-Pr	4-Me_2N-C_6H_4

Scheme 2. Synthesis of 5-ylidene-4-R-aminothiazol-2(5*H*)-ones. Reagents and conditions: (*i*) – 4-thioxo-2-thiazolidinone (1.0 equiv), NH_2R (1.0 equiv), EtOH, reflux, 1 h; (*ii*) – 4-R-aminothiazol-2(5*H*)-one (**II**) (1.0 equiv), R^1CHO (1.0 equiv), AcONa (1.0 equiv), AcOH, reflux, 3 h.



Scheme 3. Possible amino-imino tautomeric forms of target compounds.

substituent at the exocyclic nitrogen atom) are characterized by amino-imino tautomerism, and imino form can exist in the *Z*-and *E*-tautomeric forms [20]. Analogous we can assume the existence of such tautomerism for target 4-aminothiazol-2(5*H*)-ones (Scheme **3**).

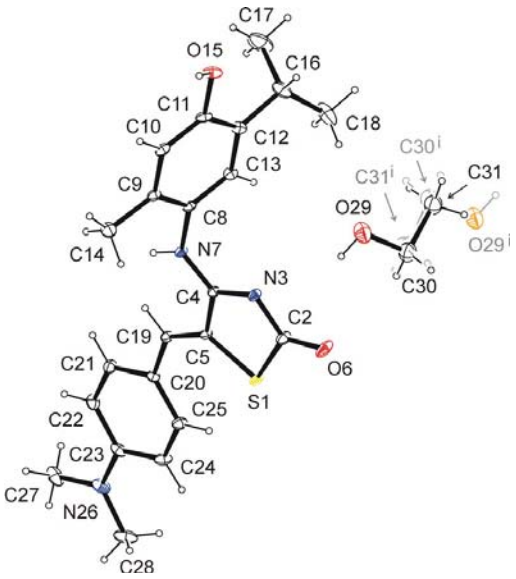


Fig. (2). The molecules of **IIIq** and ethanol showing the atomic labelling scheme. Non-H atoms are drawn as 30% probability displacement ellipsoids and H atoms are drawn as spheres of an arbitrary radius. Symmetry code: (i) 1-*x*,1-*y*,1-*z*.

In the ^{1}H NMR spectra of 4-arylaminothiazol-2(5*H*)-ones signals only one tautomeric form were observed. The signal of exocyclic NH-group appears as a singlet at 10.00-11.79 ppm. To clarify structure of the target compounds the X-ray analysis of **IIIq** was carried out and existing of the amino form was confirmed.

The crystal structure of 5-(4-dimethylaminophenylmethylidene)-4-(4-hydroxy-5-isopropyl-2-methylphenylamino) thiazol-2(5*H*)-one (**IIIq)** has a form of an ethanol solvate with the molar ratio 3 : 1 of the solute to solvent (Fig. **2**).

According to this observation, statistically every third ethanol molecule in the crystal lattice is missing and the centrosymmetry of the lattice formed by solvent is disturbed. Between solvent and solute molecules only week O29–H29···O6, O29–H29···N3 intermolecular contacts are observed (Table **1**). For this reason, the investigated compound also could be classified as an inclusion compound.

Compound **IIIq** can occur in three tautomeric forms presented in Fig. (**3**).

The results of the X-ray analysis indicate that the hydrogen atom is located at N7 position which is in agreement with the structure containing a carbonylimino group in the thiazolidin-2-one moiety and an exocyclic amine nitrogen (form **IIIq-1** (first one) (Figs. **2** and **3**). The presence of the hydrogen atom at N7 and its absence at N3 position are confirmed by the hydrogen bonds N7–H7···O6^{i} and O15–H15···N3^{ii} with the N7 atom acting as a proton donor and N3 atom as a proton acceptor (Fig. **4**, Table **1**).

Table 1. Hydrogen-bonding geometry (Å, º).

D– H···*A*	*D*– H	H···*A*	*D*···*A*	*D*– H···*A*
N7–H7···O6^{i}	0.828(17)	2.025(18)	2.8214(17)	161.2(19)
O15–H15···N3^{ii}	0.85(3)	1.97(3)	2.792(2)	160.5(19)
O29–H29···O6	0.82	2.44	3.157(4)	147
O29–H29···N3	0.82	2.46	3.210(4)	153

Symmetry codes: (i) 1.5-*x*,0.5+*y*,1.5-*z* ; (ii) 1.5-*x*,1.5-*y*,1-*z*

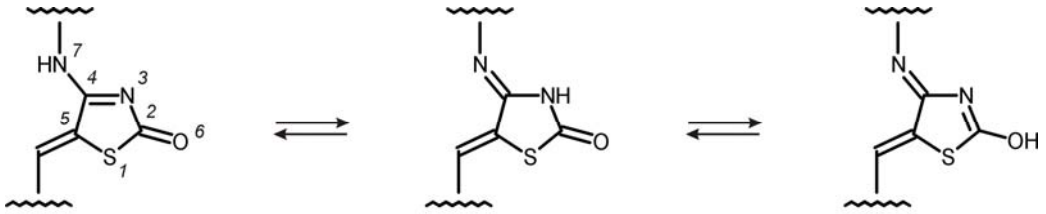


Fig. (3). Possible tautomeric structures of **IIIq**.

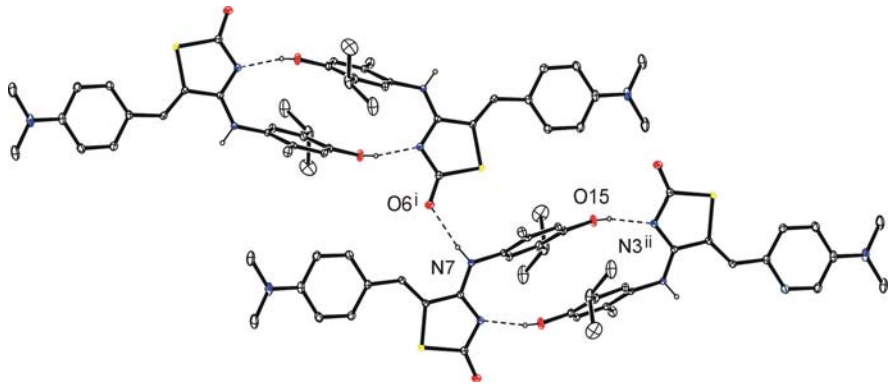


Fig. (4). Hydrogen bonds linking solute molecules (**IIIq**) to each other. For symmetry codes see Table **1**. H atoms not involved in hydrogen-bonding have been omitted for clarity.

The bond distances N3=C4 [1.3336(16) Å] and C4–N7 [1.3301(16) Å] are comparable what is typical observation for this tautomeric form. They are close to the mean values for the bonds *(O*=*C*-)N=C[1.325(1)Å] and (*S,N*-)C–NH [1.315(2) Å], acquired from 20 structures containing 2-aminothiazol-4(5*H*)-one moiety that are accessible from the CSD Cambridge, Version 5.35 [38]. The CSD revealed no crystal structures of 4-aminothiazol-2(5*H*)-one derivatives. It can be remarked that bond distances N3=C4 and C4–N7 both reveal partially double character. They are shortened by about 26 and 28σ with respect to the mean value of the single bond length Csp^2–N, 1.383(1) Å, obtained from 117 N3-substituted 2-imino-4-thiazolidinones (CSD, Cambridge, Version 5.35 [38]). On the other hand, they are lengthened by about 29 and 27σ in comparison with the literature double C=N bond length {1.279(1) Å [39]}. The observed partial double character of the C4–N7 bond is the cause of the hampered rotation and synperiplanar orientation of the N3–C4 and N7–C8 bonds [torsion angle N3–C4–N7–C8: 7.1(2)°]. The observed interatomic C5–C19 distance {1.3518(18) Å} confirms the presence of a double bond between these atoms. The torsional angle S1–C5–C19–C20, 2.6(2)°, indicates a *Z*-configuration of *p*-dimethylaminophenyl group. The phenyl ring of this substituent is almost coplanar with the thiazolidin-2-one system – the dihedral angle is merely 4.32(5)°. The other phenyl ring present in the molecule, belonging to 2-methyl-4-hydroxy-5-isopropylphenylamine substituent, is inclined to the thiazolidin-2-one system at much higher angle, *i.e.* 68.89(5)°.

Evaluation of Anticancer Activity in vitro

The anticancer activity screening of target compounds was performed within *Developmental Therapeutic Program* (National Cancer Institute USA (NCI) – www.dtp.nci.nih.gov) according to the procedure described elsewhere [40-42] and included several phases. At the first phase the compounds were estimated at one-dose primary anticancer assay towards: *i)* three cell lines panel (the human tumor cell lines: *MCF7* – breast cancer, *NCI-H460* – lung cancer and *SF-268* – CNS cancer) at concentration 10^{-4}M, *ii)* sixty cell lines panel (the human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers) at concentration 10^{-5}M. In the screening assay, each cell line was inoculated and preincubated for 24-48 h on a microtiterplate. Then tested compounds were added at the mentioned con-

Table 2. Anticancer activity towards 60 cancer cell lines (10^{-5}M).

Comp.	Mean Growth, %	Range of Growth, %	The Most Sensitive Cell Line / Cancer Type	Growth of the Most Sensitive Cell Lines, %
Ii	93.66	28.33 to 176.64	*UACC-257*/M	28.33
IIIc	93.10	-2.47 to 139.94	*NCI-H522*/nscL *MCF-7*/BC *MDA-MB-468*/BC	-2.47 43.53 31.84
IIIf	94.56	15.53 to 150.34	*CCRF-CEM*/L *RPMI-8226*/L *NCI/ADR-RES*/OC *MDA-MB-468*/BC	46.78 46.72 44.87 15.53
IIIg	101.91	68.10 to 130.09	*SR*/L	68.10
IIIh	98.79	11.68 to 210.62	*KM12* /CC *NCI-H322M*/nscL *MALME-3M*/M	11.68 44.83 33.35
IIIi	91.76	66.00 to 158.14	*HCT-116*/CC	66.00
IIIk	60.57	-34.41 to 125.74	*HCT-116*/CC *KM12*/CC *SNB-75*/CNS *U251*/CNS *OVCAR-3*/OC *786-0*/RC *TK-10*/RC *MDA-MB-468*/BC	21.38 16.78 -18.79 29.05 -34.41 1.06 -1.52 6.02
IIIl	60.21	-82.10 to 125.52	*HL-60(TB)*/L *NCI-H522*/nscL *SF-539*/CNS *OVCAR-4*/OC *UO-31*/RC *MCF-7*/BC *HS 578T*/BC *T-47D*/BC	-9.34 -49.46 -9.47 16.90 -82.10 8.37 -16.80 -10.70
IIIm	98.64	78.39 to 114.26	*T-47D*/BC	78.39
IIIp	75.33	-15.52 to 144.56	*HOP-92*/nscL *SF-295*/CNS *RXF 393*/RC *HS 578T*/BC	-15.52 5.64 25.65 -10.20

Hereinafter: M – Melanoma; nscL – Non-Small Cell Lung Cancer; CNS – CNS Cancer; RC – renal cancer; L – leukemia; BC – breast cancer; OC – Ovarian Cancer; CC – Colon Cancer.

centration and the culture was incubated for further 48 h. Sulforhodamine B (SRB) were used as endpoint in a cytotoxicity assay. Results of screening assay were presented as growth percent (GP) of the treated cells when compared to the untreated control cells. The compounds tested in three cell line assay (**Ia-Id**, **Ig**, **IIIa**, **IIIb**, **IIIj** and **IIIn**) didn't exhibit the significant anticancer effect (GPs – more than 32%), except of compound **IIIa** (GP for *MCF7* = 22%, *NCI-H460* =31% and *SF-268*= 62%), which was selected for further investigation. The results of 10^{-5}M assay (**Ii**, **IIIc**, **IIIf-i**, **IIIk-m**, **IIIp**) are shown in Table **2**.

Tested compounds possessed the moderate to low anticancer activity, as indicated by the average values (all 60 tested cancer cell lines). However the significant specific effect on certain cancer cell lines had been found: stimulation

Table 3. Anticancer activity against 60 tumor cell lines (10-fold dilutions/ five concentrations).

Comp.	MG_MID/range			The most Sensitive Cell Lines	GI_{50} (μM)	TGI (μM)	LC_{50} (μM)
	pGI_{50}	pTGI	pLC_{50}				
IIIa	4.58/ 1.57	4.09/ 0.66	4.01/ 0.17	*CCRF-CEM*/L	2.68	>100	>100
				MOLT-4/L	15.9	>100	>100
				RPMI-8226/L	16.2	>100	>100
				HCT-116/CC	17.6	34.5	67.5
				MALME-3M/M	13.3	75.0	>100
				OVCAR-5/OC	18.2	51.6	>100
				A498/RC	5.61	21.7	71.6
				TK-10/RC	16.1	5.47	>100
				DU-145/PC	14.9	33.0	73.0
				T-47D/BC	13.7	55.0	>100
IIId	4.27/ 2.00	4.02/ 0.64	4.00/ 0.14	*CCRF-CEM*/L	0.99	>100	>100
				RPMI-8226/L	3.19	>100	>100
				SR/L	3.19	23.1	73.0
				KM-12/CC	6.19	>100	>100
				U-251/CNS	7.51	27.2	82.9
				CAKI-1/RC	6.63	>100	>100
IIIe	4.09/ 1.77	4.00/ 0.16	4.00/ 0.0	*CCRF-CEM*/L	4.05	>100	>100
				HOP-92/nscL	1.68	68.9	>100
				SNB-75/CNS	23.7	83.7	>100
				U251/CNS	19.2	>100	>100
IIIk*	4.73/ 1.81 4.96/ 2.66*	4.12/ 1.45 4.25/ 1.33*	4.03/ 1.13 4.07/ 0.95*	*SR*/L	3.53/3.29*	>100/23.5*	>100/>100*
				RPMI-8226/L	-/5.24	-/>100	-/>100*
				A549/ATCC/nscL	5.55/4.80*	>100/>100*	>100/>100*
				HOP-92/nscL	2.65/4.80*	27.4/51.0*	>100/
				NCI-H460/nscL	4.08/8.21*	>100/>100*	>100/>100*
				HCT-116/CC	3.81/3.06*	>100/18.2*	>100/>100*
				SF-539/CNS	1.88/2.54*	5.03/9.30*	19.1/31.0*
				SNB-75/CNS	1.68/2.12*	3.53/6.05*	7.41/39.8*
				U251/CNS	1.85/1.90*	4.46/4.72*	>100/31.5*
				OVCAR-3/OC	2.55/2.11*	-/4.66	>100/11.2*
				OVCAR-4/OC	-/2.89	-/>100	-/>100
				786-0/RC	2.09/2.74*	-/10.5*	>100/42.6*
				A498/RC	2.40/6.54*	-/23.1*	>100/58.1*
				RFX 393/RC	5.75/2.92*	29.5/11.3*	>100/45.0*
				TK-10/RC	1.94/5.48*	4.32/24.6*	-/96.6
				MCF-7/BC	1.54/0.83*	>100/>100*	>100/>100
				MDA-MB-468/BC	-/0.22	-/>100	-/>100
IIIl	4.09/ 2.50	4.02/ 0.83	4.00/ 0.00	*MCF7*/BC	0.315	>100	>100
				T-47D/BC	1.46	14.9	>100
IIIo	4.71/ 1.04	4.29/ 0.66	4.04/ 0.27	*HOP-92*/nscL	4.89	22.1	66.4
				OVCAR-3/OC	10.1	27.2	73.5
				BT-549/BC	10.0	33.5	>100
IIIp	5.02/ 3.73	4.27/ 1.29	4.04/ 0.48	*CCRF-CEM*/L	3.06	>100	>100
				K-562/L	1.99	21.0	>100
				RPMI-8226/L	2.41	>100	>100
				NCI-H226/ncsL	2.11	9.10	45.6
				HCT-116/CC	2.60	14.4	41.0
				HCT-15/CC	2.51	46.9	>100
				RXF 393/RC	0.01	5.18	33.2

* - data of double assay; MG_MID (mean graph midpoint) – average level of all tested cell lines

influence on cells growth (GP > 100%), and even cells death (GP < 0%). Despite the sensitivity of lines representing different cancer types, the trend of some cell lines (namely – *UO-31*/RC*, MDA-MB-468*/BC, *NCI-H522*/nscL, *KM12*/CC) sensitivity to the thiazolidinone derivatives from different chemical groups was observed [3, 20-22, 24-27, 29].

Three compounds (**IIIa**, **IIIl**, **IIIp**) tested at single concentration, and **IIId**, **IIIe**, **IIIk**, **IIIo** were screened towards a sixty tumor cell lines panel at 5 different concentrations (100μM, 10μM, 1μM, 0.1μM and 0.01μM) [40-43]. The same screening protocol was used and GPs were evaluated for each cell lines at each compounds concentrations. Three dose-dependent parameters – GI_{50} (molar concentration of the compound that inhibits 50% net cell growth); TGI (molar concentration of the compound leading to total inhibition of cell growth) and LC_{50} (molar concentration of the compound leading to 50% net cell death) were calculated and presented as pGI_{50}, pTGI, pLC_{50} for each cell lines (Table **3**). In the case if the activity level was not reached or was exceeded, the parameter value was expressed as more or less than the maximum or minimum concentration tested. Besides, a mean graph midpoints (MG_MID) were calculated (for the calculation insensitive cell lines are included with the highest concentration tested) for each of the parameters, representing an average activity parameter over all cell lines for each compound.

The comparison of anticancer activity patterns allow to outline some features of structure-activity relationships among tested compounds. In general 4-substituted-amino derivatives possessed the more pronounced antitumor potential, while the compounds with unsubstituted amino group almost did not possess antitumor effect. The complications of ylidene fragment apparently can be treated as the optimization direction of such compounds (comparison of **Ii** & **IIIh**), that is consistent with previously data [20-22, 24, 25, 27, 29, 33, 36]. The compounds with furan moiety at C5 position are more effective when comparing the activity of **IIIk** and **IIIl** isosteres (Tables **2** and **3**). Significant is the presence/nature of substituents in the benzene ring of 4-amino moiety (compound **Ig**, **IIIb**, **IIIj** and **IIIl** and **IIIc**, **IIIk**); moreover the 4-chlorophenylamino dertivatives **IIIl** & **IIIk** are characterized by the higher activity levels. Similarly, comparision of compounds containing fluorine or chlorine atoms in arylidene fragment (**IIId** & **IIIi**) showed more efficiency of 4-chlorophenylmethylidene derivative. The analysis of cancer cell lines sensitivity allowed distinguishing a number of cell lines whose sensitivity to the tested compounds action was most expressed – *CCRF-CEM*/L, *RPMI-8226*/L, *U251*/CNS, *RFX 393*/RC, *OVCAR*/OC (Table **3**). Among the tested compounds (*Z*)-5-(furan-2-ylmethylidene)-4-(4-chlorophenylamino)thiazol-2(5*H*)-one (**IIIk**) and (*Z*)-5-(4-diethylaminophenylmethylidene)-4-(4-hydroxy-5-isopropyl-2-methylphenylamino)thiazol-2(5*H*)-one (**IIIp**) possessed the highest levels of activity. Following our previous study [20-22], the comparison with structurally related 2-aminothiazolidinone derivatives suggests the advantage of the latter as potential anticancer agents. For example, **IIId** and **IIIe** compared with isomeric compounds – 5-(4-chlorophenylmethylidene)-2-(4-hydroxyphenylamino)thiazol-4(5*H*)-one and 5-(4-fluorophenylmethylidene)-2-(4-hydroxyphenylamino)thiazol-4(5*H*)-one [20] have a less expressed effects. But formation of robust conclusions requires the large database analysis.

COMPARE Analysis

For predicting of tested compounds possible mechanism of anticancer action, the COMPARE analysis was carried out based on the NCI web-resources (http://dtp.nci.nih.gov/docs/compare/compare.html). The basis of this method is comparison of selectivity patterns (mean graph fingerprints) of tested compound with standard anticancer agents, NCI active synthetic compounds and natural extracts. Such comparison of the patterns of differential cell lines growth inhibition (Pearson correlation coefficient (PCC) is used for quantitatively expression of similarity of pattern to that of the seed) can reflect the possible mechanism of the cytotoxic action of tested compounds. Significant correlation (PCC > 0.6) of the data pattern with standard agent indicates the compound may possess the same mechanism of action; if the activity pattern does not correlate, the compound most probably has a novel mechanism of action [44, 45]. "Standard COMPARE analysis" was performed at GI_{50} LC_{50} and TGI levels for most active compounds. Obtained correlation coefficients didn't allow to distinguish cytotoxicity mechanism of tested compounds with high probability. Nevertheless moderate correlations with alkylation agents (Table **4**) were found.

It should be noted that no significant correlation with fluorodopan (NSC73754, alkylating agent), or with S-trityl-L-cysteine (NSC83265 aminoacyl-tRNA synthetases inhibitor) were detected as in the case of the related compounds [20-22, 25]. In the case of synthetic agents (Table **5**) significant correlations were found. These findings most probably can be used in the SAR analysis and development of structure optimization of target compounds.

CONCLUSION

In the present paper, the synthesis of 4-aminothiazol-2(5*H*)-one derivatives are described. Based on the structural investigation, the absence of amino-imino tautomerism and presence of only amino form were confirmed whereas related thiazolidinone derivatives were previously treated as 4-amino and 4-imino derivatives. Anticancer activity screening showed that the tested compounds possessed low to moderate potency of anticancer activity but, with the significant specific influence on some cancer cell lines. 5-Ylidene-4-R-aminothiazol-2(5*H*)-ones are more prominent in the comparison with unsubstituted analogues. (*Z*)-5-(Furan-2-ylmethylidene)-4-(4-chlorophenylamino)thiazol-2(5*H*)-one & (*Z*)-5-(4-diethylaminophenylmethylidene)-4-(4-hydroxy-5-isopropyl-2-methylphenylamino)thiazol-2(5*H*)-one possess the highest levels of anticancer activity.

EXPERIMENTAL PART

Chemistry

Materials and Methods

The starting 4-thioxo-2-thiazolidinone [53] and 4-aminothiazol-2(5*H*)-one [12] were obtained according to described previously methods. Melting points of the synthesized compounds are uncorrected and have been measured using BŰCHI B-545 melting point apparatus in open capillary

Table 4. Results of COMPARE analysis to the standard agents.

Comp.	PCC*	Standard Agent, NCS Number	CCC[#]	Seed SD	Target SD	Target's Mode of Action
GI_{50} level						
IIId	0.643	D-tetrandrine, 77037	51	0.512	0.599	calcium channel blocker [46]
IIIe	0.659	piperazine alkylator, 344007	55	0.324	0.613	alkylating agent [47]
IIIl	0.559	tamoxifen , 180973	55	0.414	0.162	estrogen receptor antagonist
IIIo	0.552	pentamethylmelamine, 118742	55	0.164	0.273	alkylating agent [48]
LC_{50} level						
IIIk	0.659	soluble Baker's Antifol, 139105	57	0.173	0.061	antimetabolite [49]
	0.599	rhizoxin, 332598	56	0.174	0.515	antimitotic agent
TGI level						
IIId	0.71	N,N-dibenzyldaunomycin, 268242	57	0.115	0.145	topoisomerase II inhibitor [50]
	0.69	cyclodisone, 348948	58	0.114	0.115	alkylating agent [51]
IIIl	0.571	methyl-GAG, 32946	54	0.111	0.112	[52]

* PCC ≥ 0.55 were selected; [#] hereinafter: CCC – Count Common Cell Lines, SD – strandard deviation

Table 5. Results of COMPARE analysis to the synthetic agents.

Comp.	PCC*	Synthetic Compound, NCS[#]	CCC	Seed SD	Target SD
GI_{50} level					
IIIk	0.686	638630	42	0.710	0.166
	0.738	631907	44	0.572	0.261
	0.712	740241	52	0.577	0.239
IIIp	0.794	630895	42	0.586	0.154
	0.792	662569	43	0.587	0.104
	0.781	azadiradione 640465	46	0.580	0.152
	0.780	645086	41	0.588	0.077
	0.780	629009	46	0.568	0.058
LC_{50} level					
IIIk	0.898	711104	58	0.109	0.062
	0.842	711222	59	0.143	0.187
	0.826	618857	56	0.146	0.109
	0.81	626387	41	0.168	0.125
	0.809	709928	56	0.146	0.070

Table 5. Contd……

Comp.	PCC*	Synthetic Compound, NCS[#]	CCC	Seed SD	Target SD
IIIp	0.773	628009	43	0.121	0.062
	0.736	652263	41	0.124	0.078
	0.736	627695	49	0.105	0.060
	0.714	678524	43	0.100	0.631
TGI level					
IIIk	0.762	715671	57	0.376	0.112
	0.751	gardenin, 94889	58	0.374	0.296
	0.734	633397	50	0.395	0.134
	0.729	717566	59	0.372	0.213
	0.706	711100	51	0.394	0.184
	0.706	652037	46	0.402	0.165
	0.704	633203	49	0.399	0.147
IIIp	0.756	743220	57	0.318	0.417

* PCC ≥ 0.65 were selected; [#] the compound structures can be found at http://dtp.nci.nih.gov/

tubes. Perkin-Elmer 2400 CHN analyzer have been used for the elemental analyses (C, H, N); the data 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 100MHz in DMSO-d_6 using tetramethylsilane as an internal standard. Chemical shifts are given in ppm units (δ scale). Mass spectra were recorded on Agilent 1100 Series LCMS apparatus (electrospray ionization (ESI).

General Procedure for Synthesis of 5-arylidene-4-aminothiazol-2(5H)-ones (Ia-i)

A mixtures of 4-aminothiazol-2(5*H*)-one (4 mmol), appropriate aldehyde (4 mmol) and anhydrous sodium acetate (4 mmol) were refluxed for 3 h in 10 mL of acetic acid. After cooling the formed precipitated was filtered off, washed with acetic acid, water, methanol, diethyl ether and re-crystallized with DMF : ethanol mixture (1:2).

(Z)-5-(4-Hydroxyphenylmethylidene)-4-aminothiazol-2 (5H)-one ***(Ia)***. Yield 71%, mp 268-270°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.93 (d, 2H, *J* = 8.0 Hz, arom.), 7.41 (d, 2H, *J* = 8.0 Hz, arom.), 7.77 (s, 1H, CH=), 8.85 (s, 1H, NH_2), 9.02 (s, 1H, NH_2), 10.23 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.6, 175.8, 159.5, 131.9, 128.6, 125.1, 124.5, 116.4. LCMS (ESI+) m/z 221 $(M + H)^+$. Calcd. for $C_{10}H_8N_2O_2S$: C, 54.53; H, 3.66; N, 12.72; Found: C, 54.20; H, 3.50; N 12.80%.

(Z)-5-(4-Hydroxy-3-methoxyphenylmethylidene)-4-aminothiazol-2(5H)-one ***(Ib)***. Yield 70%, mp 250-252°C, lit. 266-267 [54] ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.82 (s, 3H, CH_3), 7.01 (d, 1H, *J* = 8.4 Hz, arom.), 7.02 (d, 1H, *J* = 8.3 Hz, arom.), 7.06 (s, 1H, arom.), 7.77 (s, 1H, CH=), 8.82 (s, 1H, NH_2), 9.02 (s, 1H, NH_2), 9.88 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.5, 175.8, 149.0, 148.0, 128.9, 125.5, 124.7, 124.3, 116.2, 112.9, 55.6. LCMS (ESI+) *m/z* 251 $(M+H)^+$. Calcd. for $C_{11}H_{10}N_2O_3S$: C, 52.79; H, 4.03; N, 11.19; Found: C, 52.70; H, 4.15; N 11.00%.

(Z)-5-(3,4-Dimethoxyphenylmethylidene)-4-aminothiazol-2(5H)-one ***(Ic)***. Yield 75%, mp 261-263°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.81 (s, 3H, CH_3), 3.82 (s, 3H, CH_3), 7.06 (s, 1H, arom.), 7.13 (s, 2H, arom.), 7.80 (s, 1H, CH=), 8.87 (s, 1H, NH_2), 9.09 (s, 1H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.3, 175.7, 150.5, 149.0, 128.5, 126.7, 126.0, 123.8, 112.2, 55.8, 55.5. LCMS (ESI+) *m/z* 265 $(M+H)^+$. Calcd. for $C_{12}H_{12}N_2O_3S$: C, 54.53; H, 4.58; N, 10.60; Found: C, 54.60; H, 4.60; N 10.80%.

(Z)-5-(4-Chlorophenylmethylidene)-4-aminothiazol-2(5H)-one ***(Id)***. Yield 73%, mp 265-267°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.54 (d, 2H, *J* = 8.4 Hz, arom.), 7.60 (d, 2H, *J* = 8.0 Hz, arom.), 7.83 (s, 1H, CH=), 9.03 (s, 1H, NH_2), 9.28 (s, 1H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.8, 175.6, 134.4, 133.2, 131.2, 129.8, 129.5, 126.7. LCMS: *m/z* 239/241$(M+H)^+$. Calcd. for $C_{10}H_7ClN_2OS$: C, 50.32; H, 2.96; N, 11.74 Found: C, 50.40; H, 3.05; N 11.70%.

(Z)-5-(4-Diethylaminophenylmethylidene)-4-aminothiazol-2(5H)-one ***(Ie)***. Yield 84%, mp 250-252°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.12 (t, 6H, *J* = 6.7 Hz, 2xCH_3), 3.40 (d, 4H, *J* = 6.86, 2*CH_2), 6.79 (d, 2H, *J* = 8.6 Hz, arom.), 7.36 (d, 2H, *J* = 8.6 Hz, arom.), 7.71 (s, 1H, CH=), 8.65 (s, 1H, NH_2), 8.80 (s, 1H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.8, 175.7, 148.8, 132.0, 129.3, 120.7, 120.3, 111.63, 43.9, 12.5. LCMS (ESI+) *m/z* 276 $(M+H)^+$. Calcd. for $C_{14}H_{17}N_3OS$: C, 61.06; H, 6.22; N, 15.26; Found: C, 61.15; H, 6.10; N, 15.40%.

(Z)- 5-(4-Fluorophenylmethylidene)-4-aminothiazol-2(5H)-one ***(If)***. Yield 71%, mp >250°C. ^{1}H NMR (400 MHz,

DMSO-d_6): δ 7.39 (t, 2H, J = 8.1 Hz, arom), 7.57-7.60 (m, 2H, arom), 7.85 (s, 1H, CH=), 9.0 (s, 1H, NH_2), 9.22 (s, 1H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.0, 175.7, 162.6 (d, J = 250 Hz), 131.9 (d, J = 9 Hz), 130.9, 128.7, 127.0, 116.5 (d, J = 22 Hz). LCMS (ESI +) *m/z* 223 $(M+H)^+$. Caldc. for $C_{10}H_7FN_2OS$: C, 54.05; H, 3.17; N, 12.60%. Found: C, 54.00; H, 3.25; N 12.50%.

*(Z)-5-(Thiophen-2-ylmethylidene)-4-aminothiazol-2(5H)-one (**Ig**)*. Yield 71%, mp >250°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.15 (s, 1H, arom.), 7.33 (s, 1H, arom.), 7.83 (s, 1H, arom.), 8.02 (s, 1H, CH=), 8.70 (s, 1H, NH_2), 8.85 (s, 1H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.0, 175.3, 139.0, 132.6, 132.3, 128.9, 126.8, 121.5. LCMS (ESI+) *m/z* 211 $(M+H)^+$. Calcd. for $C_8H_6N_2OS_2$: C, 45.70; H, 2.88; N, 13.32; Found: C, 45.80; H, 2.30; N, 13.20%.

*(Z)-5-(Furan-2-ylmethylidene)-4-aminothiazol-2(5H)-one (**Ih**)*. Yield 65%, mp 232-236°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.73 (m, 1H, arom.), 6.86 (d, 1H, J = 3.4 Hz, arom.), 7.74 (s, 1H, arom.), 8.00 (s, 1H, CH=), 8.88 (s, 1H, NH_2), 9.08 (s, 1H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.6, 175.1, 149.8, 146.8, 126.3, 116.3, 115.2., 113.5. LCMS (ESI +) *m/z* 195 $(M+H)^+$. Calcd. for $C_8H_6N_2O_2S$: C, 49.48; H, 3.11; N, 14.42; Found: C, 49.60; H, 3.20; N, 14.20%.

*(Z)-5-[4-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)phenylmethylidene]-4-aminothiazol-2(5H)-one (**Ii**)*. Yield 67%, mp 126-128°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.08 (dd, 1H, J = 15.6, 3.2 Hz, CH_2CH), 3.92 (dd, 1H, J = 15.2, 8.4 Hz, CH_2CH), 5.35 (dd, 1H, J = 8.4, 3.2 Hz, CH_2CH), 6.66 (t, 1H, J = 8.1 Hz, arom), 6.97 (d, 2H, J = 8.3 Hz, arom.), 7.08 (t, 2H, J = 8.2 Hz, arom), 7.20-7.38 (m, 9H, arom), 7.68 (s, 1H, NH_2), 7.72 (s, 1H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 147.3, 144.3, 142.7, 132.4, 129.1, 129.0, 128.8, 128.7, 127.5, 126.0, 125.8, 118.7, 113.0, 63.3, 43.1. Calcd. for $C_{25}H_{20}N_4OS$: C, 70.73; H, 4.75; N, 13.20; Found: C, 70.80; H, 4.60; N 13.30%.

General Procedure for Synthesis of 5-ylidene-4-arylaminothiazol-2(5H)-ones (IIIa-p)

A mixture of 4-thioxo-2-thiazolidinone (10 mmol) and appropriate amine (10 mmol) were refluxed for1 h in 25 mL of ethanol. After cooling to the room temperature formed precipitate (4-R-aminothiazol-2(5*H*)-one (**IIa-i**) was filtered off, washed with methanol and recrystallized with appropriate solvent.

A mixture of appropriate **II** (3 mmol), aldehyde (3 mmol), and anhydrous sodium acetate (3 mmol) were refluxed for 3 h in glacial acetic acid (10 mL). Obtained precipitate was filtered off, washed with acetic acid, water and methanol and recrystallized with DMF : ethanol or DMF : acetic acid (1:2) mixtures.

*(Z)-5-(4-Hydroxyphenylmethylidene)-4-phenylaminothiazol-2(5H)-one (**IIIa**)*. Yield 78%, mp >250°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.92 (d, 2H, J = 8.0 Hz, arom), 7.20 (t, 2H, J = 8.0 Hz, arom.), 7.42 (t, 2H, J = 8.2 Hz, arom), 7.47 (d, 2H, J = 8.0 Hz, arom), 7.80 (d, 2H, J = 8.2 Hz, arom), 8.05 (s, 1H, CH=), 10.15 (s, 1H, OH), 10.54 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 178.5, 170.7, 159.8, 138.4, 132.3, 129.7, 128.9, 125.5, 125.0, 124.9, 122.5, 116.5. LCMS (ESI+) *m/z* 297 $(M+H)^+$. Calcd. for $C_{16}H_{12}N_2O_2S$: C, 64.85; H, 4.08; N, 9.45; Found: C, 64.90; H, 4.20; N 9.30%.

*(Z)-5-(Thiophen-2-ylmethylidene)-4-phenylaminothiazol-2(5H)-one (**IIIb**)*. Yield 50%, mp >250°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.22 (t, 2H, J = 8.0 Hz, arom), 7.26 (t, 1H, J = 4.2 Hz, thioph.), 7.43 (t, 2H, J = 8.0 Hz, arom.), 7.50 (d, 1H, J = 4.0 Hz, thioph.), 7.81 (d, 2H, J = 8.0 Hz, arom.), 7.94 (d, 1H, J = 4.0 Hz, thioph.), 8.44 (s, 1H, CH=), 10.67 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.9, 170.1, 138.9, 138.3, 133.4, 133.0, 129.1, 128.9, 127.0, 125.7, 122.5, 122.4. LCMS (ESI+) *m/z* 287 $(M+H)^+$. Calcd. for $C_{14}H_{10}N_2OS_2$: C, 58.72; H, 3.52; N, 9.78; Found: C, 58.80; H, 3.60; N, 9.70%.

*(Z)-5-(Furan-2-ylmethylidene)-4-phenylaminothiazol-2(5H)-one (**IIIc**)*. Yield 60%, mp >250°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.81 (m, 1H, furan.), 7.02 (d, 1H, J =4.0 Hz, furan.), 7.24 (t, 1H, J = 8.0 Hz, furan.), 7.46 (t, 2H, J = 8.0 Hz, arom.), 7.82 (d, 2H, J = 8.0 Hzm arom.) 8.09 (m, 1h, arom.), 8.11 (s, 1H, CH=), 10.72 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 78.6, 170.0, 149.7, 147.4, 147.3, 138.3, 128.9, 126.4, 125.5, 122.2, 117.3, 116.0, 113.8. LCMS (ESI+) *m/z* 271 $(M+H)^+$. Calcd. for $C_{14}H_{10}N_2O_2S$: C, 62.21; H, 3.73; N, 10.36; Found: C, 62.30; H, 3.80; N, 10.20%.

*(Z)-5-(4-Chlorophenylmethylidene)-4-(4-hydroxyphenylamino)thiazol-2(5H)-one (**IIId**)*. Yield 78%, mp >250°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.83 (d, 2H, J = 9.0 Hz, arom,) 7.57 (d, 2H, J =9.0 Hz, arom.), 7.62 (s, 4H, arom.), 8.07 (s, 1H, CH=), 9.61 (s, 1H, OH); 10.71 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.3, 170.1, 155.5, 134.5, 133.3, 131.3, 130.4, 129.7, 129.5, 126.9, 124.3, 115.3. LCMS (ESI+) m/z 331/333 $(M+H)^+$. Calcd. for $C_{16}H_{11}ClN_2O_2S$: C, 58.10; H, 3.35; N, 8.47; Found: C, 58.15; H, 3.50; N, 8.30%.

*(5Z)-5-(4-Fluorophenylmethylidene)-4-(4-hydroxyphenylamino)thiazol-2(5H)-one (**IIIe**)*. Yield 83%, mp >250°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.83 (d, 2H, J = 8.7 Hz, arom,) 7.41 (t, 2H, J =8.7 Hz, arom.), 7.52 (d, 2H, J = 8.7 Hz, arom.), 7.64-7.67 (m, 2H, arom.), 8.08 (s, 1H, CH=), 9.60 (s, 1H, OH), 10.68 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ177.5, 170.2, 162.6 (d, J = 250 Hz), 155.5, 132.1 (d, J = 9 Hz), 131.0, 131.0, 129.7, 129.4, 127.2, 124.3, 116.6 (d, J = 22 Hz), 115.3. LCMS (ESI+) *m/z* 315 $(M+H)^+$. Calcd. for $C_{16}H_{11}FN_2O_2S$: C, 61.14; H, 3.53; N, 8.91; Found: C, 61.00; H, 3.40; N, 9.00%.

*(Z)-5-(4-Hydroxyphenylmethylidene)-4-(4-hydroxyphenylamino)thiazol-2(5H)-one (**IIIf**)*. Yield 89%, mp >250°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.81 (d, 2H, J =8.4 Hz, arom.), 6.94 (d, 2H, J = 8.1 Hz, arom.), 7.47 (d, 2H, J = 8.2 Hz, arom.), 7.54 (d, 2H, J = 8.3 Hz, arom.), 8.00 (s, 1H, CH=), 9.60 (brs, 1H, OH), 10.40 (brs, 2H, OH, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 178.1, 170.4, 159.6, 155.3, 132.1, 130.0, 128.8, 125.2, 125.1, 124.2, 116.4, 115.2. LCMS (ESI+) *m/z* 313 $(M+H)^+$. Calcd. for $C_{16}H_{12}N_2O_3S$: C, 61.53; H, 3.87; N, 8.97; Found: C, 61.50; H, 3.90; N, 9.10%.

*(Z)-5-(Thiophen-2-ylmethylidene)-4-(4-hydroxyphenylamino)thiazol-2(5H)-one (**IIIg**)*. Yield 46%, mp >250°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.81 (d, 2H, J =8.5 Hz, arom.), 7.30 (t, 1H, J = 3.9 Hz, arom.), 7.50 (m, 1H, arom.), 7.56 (d, 2H, J = 8.5 Hz, arom.), 7.98 (d, 1H, J = 4.5 Hz, a-

rom.), 8.39 (s, 1H, CH=), 9.60 (brs, 1H, OH), 10.66 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.4, 169.7, 155.4, 139.0, 132.9, 132.6, 129.9, 129.0, 127.2, 124.1, 121.6, 115.3. LCMS (ESI+) *m/z* 303 $(M+H)^+$. Calcd. for $C_{14}H_{10}N_2O_2S_2$: C, 55.61; H, 3.33; N, 9.26; Found: C, 55.50; H, 3.10; N, 9.35%.

*(Z)-5-(3-Phenylallylidene)-4-(4-ethoxyphenylamino)thiazol-2(5H)-one (**IIIh**)*. Yield 84%, mp 255-258°C. ^{1}HNMR (400 MHz, DMSO-d_6): δ 1.51 (t, 3H, *J* = 6.7 Hz, CH_3), 3.94 (m, 2H, OCH_2), 7.04 (m, 1H, arom.), 7.30–7.47 (m, 6H, arom.), 7.49–7.61 (m, 2H, arom.), 7.62–7.76 (m, 4H, arom.), 10.62 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.9, 161.5, 159.1, 142.6, 136.8, 136.1, 134.4, 130.3, 129.4, 128.9, 127.9, 127.8, 127.6, 114.8, 63.7, 14.7. LCMS (ESI+) *m/z* 351 $(M+H)^+$. Calcd. for $C_{20}H_{18}N_2O_2S$: C, 68.55; H, 5.18; N, 7.99; Found: C, 68.50; H, 5.10; N, 8.10%.

*(Z)-5-(4-Chlorophenylmethylidene)-4-(4-fluorophenylamino)thiazol-2(5H)-one (**IIIi**)*. Yield 90%, mp >250°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.31 (t, 2H, *J* = 8.0 Hz, arom), 7.63 (brs, 4H, arom.), 7.81 (m, 2H, arom.), 8.11 (s, 1H, CH=), 10.86 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ177.7, 170.7, 159.6 (d, *J* = 243 Hz), 134.8, 134.6, 133.1, 131.4, 130.1, 129.6, 127.8, 124.7 (d, *J* = 9 Hz), 115.7 (d, *J* = 23 Hz). LCMS (ESI+) *m/z* 333/335 $(M+H)^+$. Calcd. for $C_{16}H_{10}ClFN_2OS$: C, 57.75; H, 3.03; N, 8.42; Found: C, 57.80; H, 2.90; N, 8.50%.

*(Z)-5-(Thiophen-2-ylmethylidene)-4-(4-fluorophenylamino)thiazol-2(5H)-one (**IIIj**)*. Yield 78%, mp 277-278°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.22 (t, 2H, *J* = 8.8 Hz, arom.), 7.29 (t, 1H, *J* = 3.6 Hz, thiophen.), 7.50 (d, 1H, *J* = 3.6 Hz, thiophen.), 7.81-7.84 (m, 2H, arom.), 7.94 (d, 1H, *J* = 4.8 Hz, thiophen.), 8.40 (s, 1H, CH=), 10.73 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.8, 170.2, 159.5 (d, *J* = 243 Hz), 138.8, 134.7, 133.2 (d, J = 44 Hz), 129.1, 126.8, 124.5, 124.4, 122.5, 115.6 (d, J = 23 Hz). LCMS (ESI+) *m/z* 305 $(M+H)^+$. Calcd. for $C_{14}H_9FN_2OS_2$: C, 55.25; H, 2.98; N, 9.20; Found: C, 55.35; H, 3.00; N, 9.10%.

*(Z)-5-(Furan-2-ylmethylidene)-4-(4-chlorophenylamino) thiazol-2(5H)-one (**IIIk**)*. Yield 68%, mp >250°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.74 (brs, 1H, furan.), 6.95 (d, 1H, *J* = 4.2 Hz, furan.), 7.44 (d, 2H, *J* = 8.8 Hz, arom.), 7.86 (d, 2H, *J* = 8.8 Hz, arom.), 7.99 (brs, 1H, furan.), 8.06 (s, 1H, CH=), 10.67 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 178.6, 169.8, 149.6, 147.5, 137.3, 129.3, 128.8, 126.1, 123.7, 117.5, 116.2, 113.8. LCMS (ESI+) *m/z* 305/307 $(M+H)^+$. Calcd. for $C_{14}H_9ClN_2O_2S$: C, 55.18; H, 2.98; N, 9.19; Found: C, 55.20; H, 3.10; N, 9.00%.

*(Z)-5-(Thiophen-2-ylmethylidene)-4-(4-chlorophenylamino)thiazol-2(5H)-one (**IIIl**)*. Yield 63%, mp >250°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.28 (t, 1H, *J* = 4.0 Hz, thiopen.), 7.45 (d, 2H, *J* = 8.0 Hz, arom.), 7.51 (d, 1H, *J* = 4.0 Hz, thiophen.), 7.86 (d, 2H, *J* = 8.0 Hz, arom.), 8.42 (s, 1H, CH=), 10.73 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.9, 170.0, 138.8, 137.3, 133.6, 133.2, 129.4, 129.1, 128.9, 126.8, 123.8, 122.8. LCMS (ESI+) m/z 321/323 $(M+H)^+$. Calcd. for $C_{14}H_9ClN_2OS_2$: C, 52.41; H, 2.83; N, 8.73; Found: C, 52.50; H, 3.00; N, 8.60%.

*(Z)-5-(4-Fluorophenylmethylidene)-4-(4-methylphenylamino)thiazol-2(5H)-one (**IIIm**)*. Yield 74%, mp 272-274°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.33 (s, 3H, CH_3), 7.26 (d, 2H, *J* = 8.39 Hz, arom.), 7.42 (t, 2H, *J* = 9.0 Hz, arom.), 7.66-7.70 (m, 4H, arom.), 8.13 (s, 1H, CH=), 10.74 (s, 1H NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.8, 170.4, 160.7 (d, *J* = 248 Hz), 135.7, 135.1, 132.2 (d *J* = 9 Hz), 131.0, 130.9, 129.3, 129.3, 127.758, 122.496, 116.6 (d, *J* = 22 Hz), 20.7. LCMS (ESI+) *m/z* 313 $(M+H)^+$. Calcd. for $C_{17}H_{13}FN_2OS$: C, 65.37; H, 4.19; N, 8.97; Found: C, 65.50; H, 4.10; N, 8.80%.

*(Z)-5-(Furan-2-ylmethylidene)-4-(3-methylphenylamino) thiazol-2(5H)-one (**IIIn**)*. Yield 86%, mp 232-233°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.34 (s, 3H, CH_3), 6.79 (d, 1H, J = 3.7 Hz, furan.), 7.00 (d, 1H, *J* = 3.6 Hz, furan.), 7.05 (d, 1H, *J* = 7.6 Hz, arom.), 7.32 (t, 1H, *J* = 8.0 Hz, arom.), 7.61 (d, 2H, *J* = 8.4 Hz, arom.), 8.07 (d, 1H, *J* = 4.0 Hz, furan.), 8.10 (s, 1H, CH=), 10.63 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 178.5, 169.9, 149.7, 147.3, 138.2, 138.1, 128.8, 126.4, 126.2, 122.6, 119.4, 117.2, 115.9, 113.8, 21.2. LCMS (ESI+) *m/z* 285 $(M+H)^+$. Calcd. for $C_{15}H_{12}N_2O_2S$: C, 63.36; H, 4.25; N, 9.85; Found: C, 63.40; H, 4.20; N, 9.95%.

*(Z)-5-(4-Chlorophenylmethylidene)-4-(2-hydroxyphenylamino)thiazol-2(5H)-one (**IIIo**)*. Yield 76%, mp 234-236°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.87 (m, 1H, arom.), 6.97 (m, 1H, arom.), 7.17 (m, 1H, thiophen.), 7.40 (m, 1H, arom.), 7.62 (brs, 4H, arom.), 8.11 (s, 1H, CH=), 9.95 (brs, 1H, OH), 10.54 (brs, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.0, 172.6, 151.5, 134.6, 133.2, 131.4, 129.9, 129.5, 128.4, 127.4, 127.2, 125.2, 119.1 116.4. LCMS (ESI+) *m/z* 331/333 $(M+H)^+$. Calcd. for $C_{16}H_{11}ClN_2O_2S$: C, 58.10; H, 3.35; N, 8.47; Found: C, 58.20; H, 3.30; N, 8.40%.

*(Z)-5-(4-Diethylaminophenylmethylidene)-4-(4-hydroxy-5-isopropyl-2-methylphenylamino)thiazol-2(5H)-one (**IIIp**)*. Yield 54%, mp 255-258°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.15 (m, 12H, 4*CH_3), 2.07 (s, 3H, CH_3), 3.18 (m, 1H, $CHMe_2$), 3.43 (q, 4H, *J* = 6.7 Hz, 2*CH_2), 6.70 (s, 1H, arom.), 6.83 (d, 2H, *J* = 8.4 Hz, arom), 6.98 (s, 1H, arom.), 7.41 (d, 2H, *J* = 8.3 Hz, arom.), 7.91 (s, 1H, CH=), 9.39 (s, 1H, OH), 10.30 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.9, 173.0, 153.5, 148.8, 132.3, 132.2, 129.3, 128.4, 124.4, 120.5, 120.3, 116.5, 111.7, 43.9, 26.3, 22.6, 17.6, 12.6. LCMS (ESI+) *m/z* 424 $(M+H)^+$. Calcd. for $C_{24}H_{29}N_3O_2S$: C, 68.05; H, 6.90; N, 9.92; Found: C, 68.00; H, 6.80; N 10.00%.

*(Z)-5-(4-Dimethylaminophenylmethylidene)-4-(4-hydroxy-5-isopropyl-2-methylphenylamino)thiazol-2(5H)-one (**IIIq**)*. Yield 68%, mp >250°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.20 (d, 6H, 2*CH_3), 2.15 (s, 3H, CH_3), 3.23 (m, 1H, $CHMe_2$), 6.75 (s, 1H, arom.), 6.98 (s, 1H, arom.), 7.49 (d, 2H, *J* = 8.4 Hz, arom), 7.53 (d, 2H, *J* = 8.4 Hz, arom.) 7.90 (s, 1H, CH=), 9.10 (s, 1H, OH), 10.30 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.4, 172.8, 152.8, 147.6, 134.8, 133.1, 131,9, 129.2, 128.6, 123.7, 120.6, 120.2, 116.0, 111.7, 41.2, 26.8, 23.4, 17.8. LCMS (ESI+) *m/z* 396 $(M+H)^+$. Calcd. for $C_{22}H_{25}N_3O_2S$: C, 66.81; H, 6.37; N, 10.62; Found: C, 66.50; H, 6.50; N 10.60%.

Crystal Structure Determination of (Z)-5-(4-diethylamino-phenylmethylidene)-4-(4-hydroxy-5-isopropyl-2-methylphenylamino)thiazol-2(5H)-one Ethanol Solvate (IIIq)

Crystal data: $C_{22}H_{25}N_3O_2S$, $1/3(C_2H_6O)$, M_r= 410.87, monoclinic, space group *C*2/*c*, *a*= 20.592(3), *b*= 13.8421(8),

c= 18.141(3) Å, β=124.42(2)º, V= 4265.7(9) Å^3, T= 130(2) K, Z= 8 (Z' = 1).

Data collection: An orange lath crystal (ethanol) of 0.42 x 0.40 x 0.08 mm was used to record 14467 (Mo$K\alpha$ radiation, θ_{max}= 29.04º) intensities on an Agilent X-calibur A diffractometer. Intensity data collection employed the ω–scans mode with "Enhance (Mo) X-ray Source". The data were corrected for Lorentz and polarization effects. Data reduction and analysis were carried out with the CrysAlis PRO program (Agilent (2011). CrysAlis PRO. Oxford Diffraction Ltd, Yarnton, England). The 5038 total unique reflections (R(int)= 0.027) were used for further calculations.

Structure solution and refinement: The structure was solved by the direct methods using the program SHELXS-97 [55] and refinement was done against F^2 for all data using SHELXL-97. The position of the H atom bonded to N atom was obtained from difference Fourier map and was refined freely. The remaining H atoms were positioned geometrically and were refined using a riding model, with C–H = 0.96 Å (CH_3), 0.97 Å (CH_2), 0.98 Å (C_{sp}3H), 0.93 Å (C_{sp}2H) and U_{iso}(H) = 1.2U_{eq}(C) or 1.5U_{eq}(C) for methyl H atoms. The methyl group was refined as a rigid group, which was allowed to rotate. The final refinement converged with R= 0.038 (for 4368 data with $I>2\sigma(I)$), wR=0.102 (on F^2 for all data), and S= 1.04 (on F^2 for all data). The largest difference peak and hole were 0.33 and -0.27 eÅ^{-3}. The molecular illustration was drawn using ORTEP-3 for Windows [56]. Software used to prepare material for publication was OLEX2 [57], WINGX [56] and PLATON [58].

The supplementary crystallographic data have been deposited at the Cambridge Crystallographic Data Centre (CCDC), 12 Union ROAD, Cambridge CB2 1EZ (UK), Tel.: (+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 982065).

Pharmacology

Anticancer screening assay was performed according to the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda, USA [40-43] at three and/or approximately sixty human tumor cell lines panel derived from nine neoplastic diseases. The cultures were incubated for 48 h after tested compound adding at single concentration (10^{-4}M (3 cell lines panel) or 10^{-5}M (60 cell lines panel). A protein binding dye (sulforhodamine B) was used for endpoint determination. The growth percents of cell lines under compounds action were evaluated spectrophotometrically versus untreated control cells and have been used as the screening results. The most active compounds in the first screening phase were tested in the 60 cancer cell lines assay at 5 different concentrations (100μM, 10μM, 1μM, 0.1μM and 0.01μM). The same 48-h protocol and an SRB protein assay were used. The growth percent was calculated at each of the compound concentrations levels as:

$(Ti - Tz) / (C - Tz)] \times 100$ (for concentrations for which $Ti \geq Tz$);

$(Ti - Tz) / Tz] \times 100$ (for concentrations for which $Ti < Tz$);

followed the seven absorbance measurements: time zero (Tz), control growth in the absence of compound (C), and test growth in the presence of tested compound at the mentioned concentration levels (Ti). Then, the next dose response parameters were calculated for each compound as: *i*) **GI_{50}** – *growth inhibition of 50%* – $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, which is the compound concentration resulting in a 50% lower net protein increase in the treated cells (measured by SRB staining) as compared to the net protein increase seen in the control cells; *ii*) **TGI** – *total growth inhibition* – $Ti = Tz$, which is the compound concentration resulting in total growth inhibition; *iii*) **LC_{50}** – concentration of compound resulting in a 50% reduction in the measured protein at the end of the compound treatment as compared to that at the beginning indicating a net loss of cells following treatment – $[(Ti - Tz)/ Tz] \times 100 = -50$. The calculations of mentioned parameter values were performed if the level of activity was reached, if the activity level was not reached or was exceeded the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested. The logGI_{50}, logTGI, logLC_{50} were then determined, defined as the mean of the log's of the individual GI_{50}, TGI, LC_{50} values. The lowest values are obtained with the most sensitive cell lines. Compounds having these values ≤4 were declared to be active.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

We are grateful to Dr. V.L. Narayanan from Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, MD, USA, for *in vitro* evaluation of anticancer activity. The authors support all people of good will currently struggling for liberty and justice in Ukraine.

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Received: July 09, 2014 Revised: January 03, 2015 Accepted: February 03, 2015