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Original article

Synthesis and *in vitro* anticancer activity of 2,4-azolidinedione-acetic acids derivatives

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ABSTRACT

The synthesis and evaluation of anticancer activity of 2,4-thia(imida)zolidinedione-3- and 5-acetic acids amides were described. The structures of compounds were determined by IR, ^{1}H NMR, and MS analysis. *In vitro* anticancer activity of these compounds has been tested in National Cancer Institute (NCI) and the relationships between structure and anticancer activity are discussed. Among 2,4-azolidinedione-acetic acids derivatives 2-[5-(4-chlorobenzylidene)-2,4-dioxo-imidazolidin-3-yl]-N-(2-trifluoromethyl-phenyl)-acetamide (**Ic**) was superior to other related compounds in terms of high selectivity for the leukemia CCRF-CEM (log $GI_{50} = -6.06$), HL-60(TB) (log $GI_{50} = -6.53$), MOLT-4 (log $GI_{50} = -6.52$) and SR (log $GI_{50} = -6.51$) cell lines.

1. Introduction

The exploration of privileged structures in drug discovery has gained significant popularity in medicinal chemistry over the past years. 4-Azolidinone (especially rhodanine, thiazolidinedione, hydantoin) derivatives are known for their broad spectrum of biological activities [1,2]. Recently, 4-azolidinones and related heterocycles were shown to be perspective as potential anticancer drug candidates [3–6].

Among 4-azolidinone derivatives the group of 4-azolidinone-3-carboxylic acids derivatives is one of the most promising. Lately, new biological effects as well as a number of biological targets for these compounds have been discovered, paving the way for drug-like substances design on their basis. For example, Epalrestat (*Z,E*-[5-(2-methyl-3-phenyl-allylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid) and its related analogues are effective aldose reductase inhibitors [7,8]. Amides of 5-arylidene-2-thioxo-4-thiazolidone-3-alkanecarboxylic and 5-(4-fluoro(chloro)phenylmethylidene)-2,4-thiazolidinedione-3-acetic acids possess marked anti-inflammatory activity [9,10]. There is an interesting fact of combined anti-inflammatory, antioxidant, and related activities for some compounds [11,12]. This is extremely important within the classical triad progression: stress–inflammation–cancer. Derivatives of 2,4-imidazolidinedione-3-alkanecarboxylic acids (which are the nitrogen-containing analogues of 4-thiazolidone-3-alkanecarboxylic acids and hydantoin derivatives) are characterized by known pharmacological potentials, the anti-arrhythmic [13,14], proteolytic [15], anticonvulsant [16–18] and antimicrobial properties in particular. 5-Substituted hydantoin-3-acetic acids derivatives are high affinity antagonists of the platelet fibrinogen receptor (GP IIb/IIIa receptor). They are a promising new class of antithrombotic agents [15].

Discovered during the last decade the anticancer activity of 4-azolidinone-3-carboxylic acids is realized via influencing the different neoplastic cells' metabolism stages [19,20] and confirmed by the discovery of antitumor effect for the substances with known pharmacological activity (antioxidant, anti-inflammatory, hypoglycaemic, immunomodulation) [21–23]. The number of molecular targets as well as some particular biological aspects for the realization of anticancer activity have been described for 4-azolidinone-3-carboxylic acids and their derivatives (Fig. 1). 5-Substituted-2-thioxo-4-thiazolidinone-3-carboxylic acids are inhibitors of protein–protein interaction of Bcl-2 and Bax family and their interaction with receptors' domains [24,25]. They are also modulators of p53 dependent pathways of apoptotic and neoplastic transformations [26] and highly active inhibitors of JSP-1 [27], which are the proteins, taking part in the processes of growth regulation, proliferation and cell-to-cell adhesion.

Also, there is a known antiproliferative potential of 5-substituted N-derivatives of hydantoin [5] and 5-arylidene-2,4-imidazolidinediones [28], which are related with inhibition of EGFR – kinase epidermal growth factor receptor. Potent necroptosis inhibitors (called necrostatins) are identified in this group of

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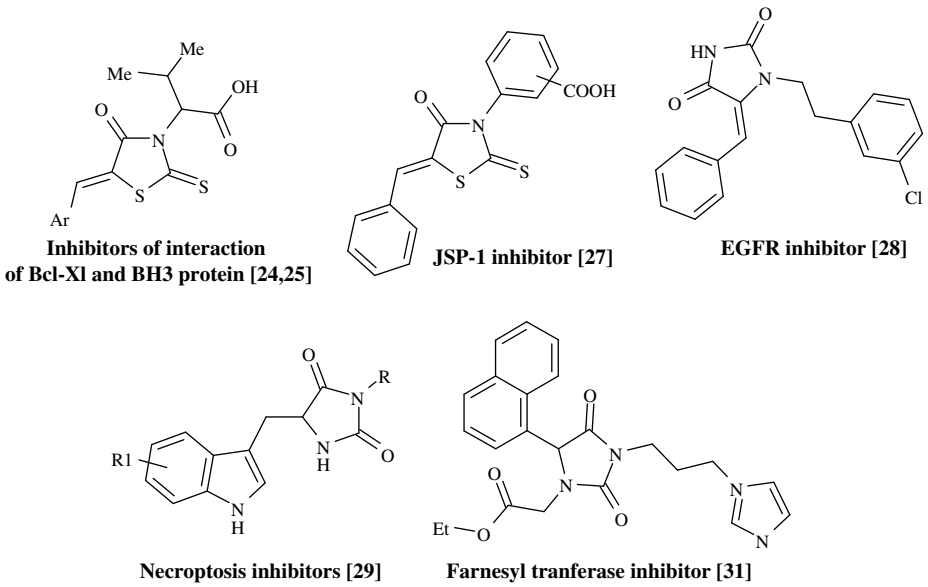


Fig. 1. Structure of 4-azolidinone derivatives with anticancer activity.

substances as well. Necroptosis is a regulated caspase-independent cell death mechanism that results in morphological features resembling necrosis, and can be induced in a FADD-deficient variant of human Jurkat T cells treated with TNF-α [29]. Hydantoin-5-acetic acid derivatives are non-hydroxamate inhibitors of TNF-α converting enzyme [30]. The latter allows considering them as potential NSAIDs and gives the possibility to identify the anticancer properties of mentioned heterocycles. Hydantoin-carboxylic acids derivatives, particularly with imidazole moiety, can inhibit Ras farnesyl transferase [31]. Ras protein plays an important role in cell growth and differentiation and needs a series of post-translational modifications including the farnesylation catalyzed by farnesyl transferase (Ftase). Therefore inhibitors of Ftase are considered as potential anticancer agents [32].

Hence, in search for more potent 4-azolidinone derivatives with anticancer activity and for structure–activity relationship we synthesized 5-arylidene-2,4-imidazolidinedione-3-acetic acids amides, their precursors and sulphur-containing analogues.

2. Results and discussion

Target compounds (I), their heterocyclic analogues and precursors are presented in Fig. 2. The study of anticancer activity of these substances (**I**–**VI**) would allow us to investigate the influence of moiety in positions 3 and 5 of core heterocycle on the realization of biological effect. Amides were selected as target compounds due to the fact of saving and increase in their pharmacological activity compared to initial acids and other derivatives [2,33].

2.1. Chemistry

Presented 2-(5-arylidene-2,4-dioxoimidazolidin-3-yl)-N-acetamide derivatives (**I**) and 2-(5-arylidene-2,4-dioxothiazolidin-3-yl)-N-acetamide derivatives (**V**) were synthesized by using known synthetic approaches [2] in several stages, which included reactions of condensation, and alkylation (Scheme 1).

5-Arylidene-2,4-azolidinediones were obtained by the Knoevenagel reaction in the medium of acetic acid in the presence of sodium acetate. The usage of hydantoin in the condensation reaction in such condition is characterized by lower yield, compared to 2,4-thiazolidinedione yields, due to lower methylene group reactivity [34]. The obtained 5-arylidene-2,4-azolidinediones were converted into appropriate potassium salts by reaction with potassium hydroxide in ethanol medium [2,35]. They were introduced thereafter into the alkylation reactions with appropriate N-chloroacetamide derivatives. It allowed obtaining target amides

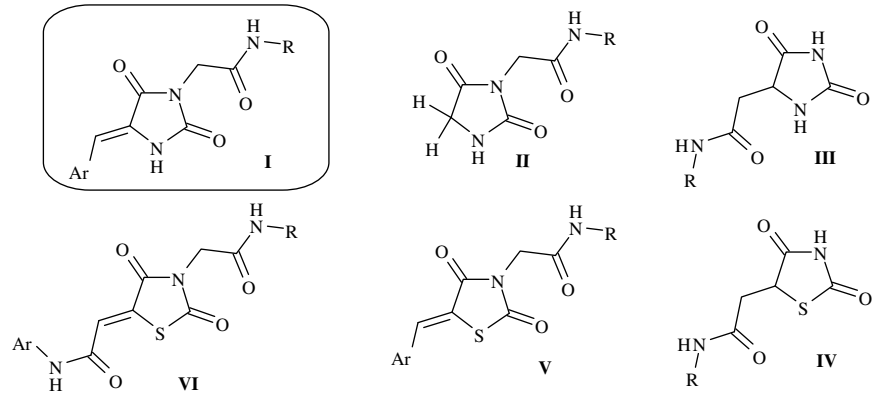
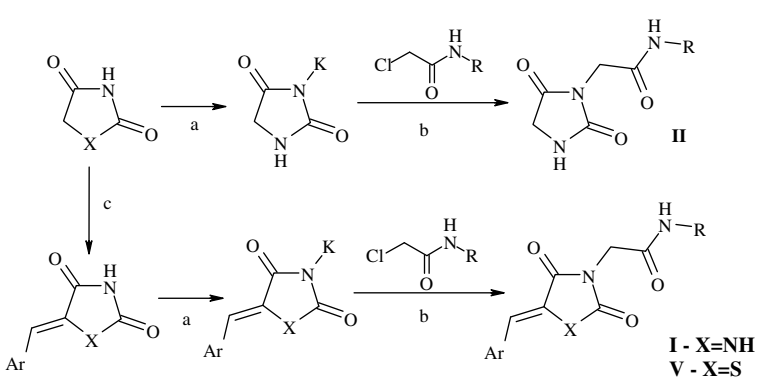


Fig. 2. Target 5-arylidene-2,4-imidazolidinedione-3-acetic acid amides **I** and their heterocyclic analogues and precursors.



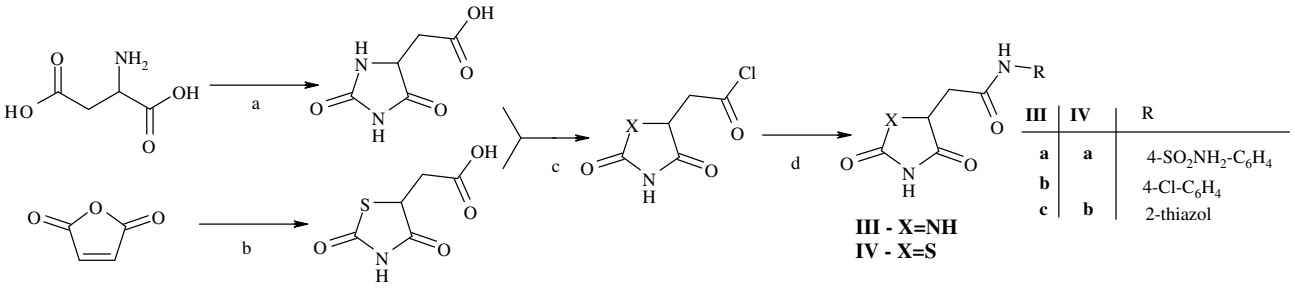
I	V	Ar	R
a	a	4-Cl-C_6H_4	3-CF_3-C_6H_4
b		4-F-C_6H_4	2-CF_3-C_6H_4
c	b	4-Cl-C_6H_4	2-CF_3-C_6H_4
d	c	4-Cl-C_6H_4	4-Cl-C_6H_4
e	d	Ph-CH=CH	4-Cl-C_6H_4
f		Ph	4-Cl-C_6H_4
g		4-OMe-C_6H_4	3-CF_3-C_6H_4
h	e	4-OMe-C_6H_4	2-thiazol
i	f	Ph-CH=CH	2-CF_3-C_6H_4
j		4-OMe-C_6H_4	2-Cl-5-CF_3-C_6H_3
k	g	4-Me_2N-C_6H_4	2-CF_3-C_6H_4
l		4-OMe-C_6H_4	4-OMe-C_6H_4

II **I - X=NH** **V - X=S**

IIa - R=3-CF_3-C_6H_4,
IIb - R=4-SO_2NH_2-C_6H_4,

a - KOH, EtOH; b - K_2CO_3, KI, DMF; c - Ar-CHO, AcONa, AcOH.

Scheme 1. Synthesis of novel 2,4-azolidinedione-3-acetic acid amides.



III	IV	R
a	a	4-SO_2NH_2-C_6H_4
b		4-Cl-C_6H_4
c	b	2-thiazol

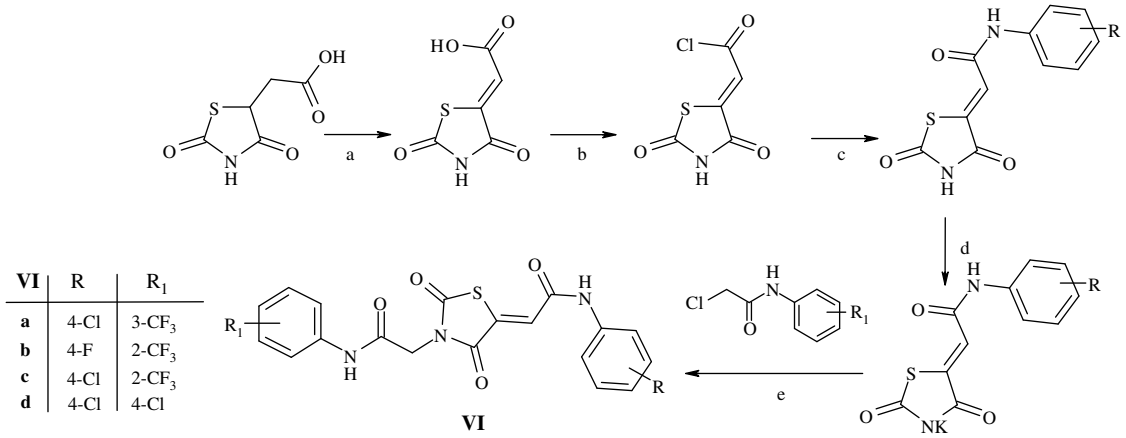
III - X=NH
IV - X=S

a - 1. KCNO, KOH 2. HCl; b - thiourea, HCl; c - $SOCl_2$, dioxane; d - R-NH_2, Et_3N, dioxane.

Scheme 2. Synthesis of 2,4-azolidinedione-5-acetic acid amides.

of 5-arylidene-2,4-azolidinedione-3-acetic acids (**I**, **V**). The reaction was performed in the DMF medium and in the presence of potassium iodide and carbonate. The described method is relatively simple, preparative and does not require additional reagent, as in case of 4-azolidinone-3-acetic acid carboxylic group functionalization [2,15]. The structures of synthesized compounds were elucidated by spectral data. The ^1H NMR spectra of compounds (**I**, **V**) showed a singlet at ~4.20–4.95 ppm of CH_2 alkyl fragment in position N-3. The chemical shift of the methylidene group of 5-arylidenederivatives (**V**) is insignificantly displaced in the weak magnetic field, $\delta \sim 8.0$ ppm, and clearly indicated that only *Z*-isomers were obtained in Knoevenagel reaction of 4-thiazolidinones with aromatic aldehydes [2,36]. Signals of NH groups appeared as single-proton singlets, particularly imidazolidine group signal is somewhat displaced in the weak magnetic field compared with exocyclic NH.

It has been previously summarized that the value and the activity tendency of mentioned compounds depended on the presence of substituent in position 5 of core cycle [2]. Therefore, we synthesized the row of 5-unsubstituted analogues of **I**. Synthesis of amides (**II**)



VI	R	R_1
a	4-Cl	3-CF_3
b	4-F	2-CF_3
c	4-Cl	2-CF_3
d	4-Cl	4-Cl

VI

a - Br_2, AcOH; b - $SOCl_2$, dioxane; c - R-C_6H_4-NH_2, Et_3N, dioxane; d - KOH, EtOH, e - K_2CO_3, KJ, DMF.

Scheme 3. Synthesis of novel 5-carboxymethylidene-2,4-thiazolidinedione-3-acetic acid diamides.

Table 1
Cytotoxic activity of the compounds in concentration 10^{-4} M against three cancer cell lines.

Test compounds	Growth of cells (%)			Activity
	NCI-H460 (Lung)	MCF7 (Breast)	SF-268 (CNS)	
Ia	**4**	**58**	**100**	**Active**
Ib	119	87	100	Inactive
Ie	**17**	**7**	**39**	**Active**
Ig	44	64	65	Inactive
IIa	106	111	113	Inactive
IIIb	121	115	114	Inactive
IIIc	108	111	114	Inactive
IVb	116	115	114	Inactive
Va	99	100	110	Inactive
Vc	101	102	105	Inactive
Vd	108	92	98	Inactive
Ve	98	98	107	Inactive
VIa	108	103	100	Inactive
VIb	77	74	75	Inactive
VIc	87	61	86	Inactive
VId	107	111	116	Inactive

Table 2
Cytotoxic activity of the compounds in concentration 10^{-5} M against 60 cell cancer lines.

Test compounds	Mean growth, %	Range of growth, %	Most sensitive cell line growth, %
If	80.27	−83.09 to 115.18	**−83.09 (UO-31/RC)** **1.51 (ACHN/RC)** **−3.51 (IGROV/OV)**
Ih	93.03	76.45–123.27	76.45 (OVCAR-5/OV)
Ii	87.24	26.61–109.05	**26.61 (RPMI-8226/L)**
Ik	90.31	31.00–127.45	**31.00 (HCT-116/ColC)**
IIb	97.16	26.03–118.54	**26.03 (UO-31/RC)**
IIIa	98.15	68.53–145.55	68.35 (SR/L)
IVa	101.62	54.24–194.65	54.24 (CAKI-1/RC)
Vb	106.35	93.46–141.74	75.85 (UO-31/RC)
Vf	95.93	62.30–138.12	62.30 (TK-10/RC)
Vg	96.45	45.18–116.01	45.81 (SR/L)

ColC – colon cancer, M – melanoma, nscLC – non-small cell lung cancer, RC – renal cancer, CNSC – CNS cancer, L – leukemia, BC – breast cancer, PC – prostate cancer, OV – ovarian cancer.

was performed in the same manner by alkylation of hydantoin potassium salt, using appropriative N-chloroacetamide derivatives. Two double-proton singlets of cyclic (∼4.00 ppm) and exocyclic (∼4.20 ppm) methylene groups were observed in ^{1}H NMR spectra. Cyclic NH-group proton is characterized by singlet at ∼8.10 ppm.

(2,4-Dioxoimidazolidin-5-yl)-acetic acid and (2,4-dioxothiazolidin-5-yl)-acetic acid were used as reagents for synthesis of isomeric to **II** 2,4-azolidinedione-5-acetic acids amides (**III**, **IV**), via conversion into acid chlorides [37], which then were used for the acylation of corresponding amines (Scheme 2).

2,4-Thiazolidinedione-5-acetic acid and their derivatives contain diastereotopic protons of methylene group. That is why the fragment $CH^AH^BCH^X$ is presented in ^{1}H NMR spectra as ABX spin system, which appears as doublet of doublets at ∼3.00 and 3.30 ppm, and ∼4.60 ppm with coupling constants $J_{AB} = 15.8$–17.4 Hz, $J_{Ax} = 6.9$–10.5 Hz, $J_{Bx} = 3.5$–5.3 Hz. High value of J_{AB} agreed with the data of Takahashi ("carbonyl effect") for structurally related 2-thioxo-4-thiazolidinone-5-acetic acids [38]. Analogous spectral pattern is observed for 2,4-imidazolidinedione-5-acetic acid. Particularly subspectrum of CH_2CH group contains two doublet doublets at ∼2.70 and 2.90 ppm and multiplet at ∼4.30 ppm with appropriate coupling constants. Exocyclic NH-group protons peaks for 2,4-thiazolidinedione-5-carboxylic amides lays in the region of 9.30–12.00 ppm, relatively to 2,4-imidazolidinedione derivatives, which spectra contain the mentioned signal at ∼7.85 ppm near aromatic protons. Cyclic NH-group proton of 2,4-thiazolidinediones is observed as broad singlet at 11.85–12.60 ppm, for 2,4-imidazilidinedione two single-proton singlets are observed in the same spectrum region as well.

The compound series (**VI**), which contain N-arylamidemethylidene fragment instead of arylidene moiety, were synthesized in order to investigate the influence of the substituent nature in position 5 on the antitumor activity realization. (2,4-Thiazolidinedione-5-ylidene)-acetic acid was used for synthesis of target 2-(2,4-dioxo-5-arylcarbamoylmethylenethiazolidin-3-yl)-N-aryl-acetamides (**VI**), which were transformed into acid chlorides and used in acylation reaction of respective amines as described previously [37]. Obtained amides were transformed into N-potassium salts and acylated by N-R-chloroacetamides as described above (Scheme 3).

Chemical shift of methylene group in position 3 of diamides (**VI**) in the ^{1}H NMR spectra were observed as singlet at ∼4.50 ppm. Methylidene fragment in position 5 also appeared as singlet at ∼7.40 ppm. Amide protons showed two singlets in the weak magnetic field within 10.00–11.30 ppm.

2.2. Biological activity

Newly synthesized compounds were selected by the National Cancer Institute (NCI) Developmental Therapeutic Program (www.dtp.nci.nih.gov) for the *in vitro* cell line screening to investigate their anticancer activity. Anticancer assays were performed according to the US NCI protocol, which was described elsewhere [39–42]. The compounds were first evaluated at one dose primary anticancer assay towards three cell lines (panel consisting of three types of human cancers: breast (MCF7), lung (NCI-H460) and CNS

Table 3
Summary of cytotoxic activity of the compounds in different concentrations (10^{-4}–10^{-8} M) towards 60 cancer cell lines.

Compounds	*N*	log GI_{50}			log TGI			log LC_{50}		
		N1	Range[a]	MG_MID	N2	Range[a]	MG_MID	N3	Range[a]	MG_MID
Ia	51	36	−8.00 to −4.03	−4.53	11	−5.16 to −4.11	−4.10	4	−4.55 to −4.05	−4.02
Ic[b]	56	8	−6.05 to −4.01	−4.26	5	−5.85 to −4.01	−4.09	3	−5.02 to −4.53	−4.05
	59	10	−7.71 to −4.10	−4.17	3	−5.51 to −4.62	−4.05	2	−4.28 to −4.09	−4.01
Id	56	53	−5,15 to −4.04	−4.57	29	−4.55 to −4.09	−4.16	6	−4.22 to −4.14	−4.01
Ie	57	20	−4.66 to −4.06	−4.13	2	−4.43 to −4.28	−4.01	1	−4.11 to −4.00	−4.00
Ig	56	16	−5.55 to −4.11	−4.12	5	−4.23 to −4.02	−4.01	0	–	−4.00
Ij	57	22	−5.57 to −4.07	−4.28	7	−4.78 to −4.04	−4.04	2	−4.31 to−4.16	−4.01
Il	57	11	−6.71 to −4.15	−4.11	1	–	−4.00	0	–	−4.00

N – number of tested human tumor cell lines; N1, N2, N3 – number of sensitive cell lines, against which the compound possessed considerable growth inhibition according to mentioned parameter (Log GI_{50}, Log TGI or Log LC_{50}) ≤ −4.00.
[a] The value >−4.00 were excluded.
[b] Data of double assay.

Table 4
The most sensitive cancer cell lines to synthesized compounds.

Compounds	Cancer type	Most sensitive cell line	Log GI_{50}	Log TGI	Log LC_{50}
Ia	Leukemia	*CCRF-CEM*	−4.87	>−4.00	>−4.00
		MOLT-4	−5.60	>−4.00	>−4.00
		RPMI-8226	−8.00	−5.16	−4.55
	Non-small cell lung cancer	*A549/ATTC*	−4.97	>−4.00	>−4.00
		HOP-62	−4.90	−4.53	−4.16
		HOP-92	−5.27	−4.54	>−4.00
		NCI-H226	−4.88	−4.33/	>−4.00
	Colon cancer	*HCT-116*	−4.86	−4.18	>−4.00
	Melanoma	*M14*	−4.84	>−4.00	>−4.00
	Renal cancer	*786-O*	−4.86	−4.45	−4.05
		ACHN	−4.84	−4.54	−4.25
		SN12C	−4.92	−4.46	−4.00
	Breast cancer	*MDA-MB-231/ATTC*	−5.34	−4.64	>−4.00
		HS578T	−4.98	−4.11	>−4.00
Ic	Leukemia	*CCRF-CEM*	−6.06	>−4.00	>−4.00
			−5.92[a]	−5.51[a]	>−4.00[a]
		HL-60(TB)	−6.53	−5.70	>−4.00
		K-562	−5.68	>−4.00	>−4.00
			−5.27[a]	>−4.00[a]	>−4.00[a]
		MOLT-4	−6.52	−5.49	−4.53
			−5.34[a]	−4.62[a]	−4.09[a]
		SR	−6.51	−5.85	−5.02
			−7.71[a]	−4.90[a]	−4.28[a]
	Non-small cell lung cancer	*HOP-92*	−4.74	−4.01	>−4.00
Id	Leukemia	*CCRF-CEM*	−4.83	−4.50	−4.17
		HL-60(TB)	−4.71	−4.33	>−4.00
		MOLT-4	−4.96	−4.55	−4.14
		RPMI-8226	−4.68	−4.17	>−4.00
		SR	−5.15	−4.47	>−4.00
	Non-small cell lung cancer	*HOP-62*	−4.82	−4.36	>−4.00
		HOP-92	−4.76	−4.38	>−4.00
		NCI-H226	−4.80	−4.35	>−4.00
	CNS cancer	*SF-268*	−4.98	−4.53	−4.07
		SF-539	−4.78	−4.41	−4.05
		SNB	−4.88	−4.44	>−4.00
		U251	−4.83	−4.53	−4.22
	Melanoma	*LOX-IMVI*	−4.81	−4.49	−4.18
	Ovarian cancer	*OVCAR-4*	−4.88	−4.19	>−4.00
		SK-OV-3	−4.71	−4.29	>−4.00
	Renal cancer	*SN-12C*	−4.77	−4.32	>−4.00
		TK-10	−4.77	−4.37	>−4.00
	Prostate cancer	*PC-3*	−4.73	−4.33	>−4.00
	Breast cancer	*MDA-MB-231/ATTC*	−4.79	−4.37	>−4.00
		HS-578T	−4.97	−4.42	>−4.00
Ie	Leukemia	*SR*	−4.47	>−4.00	>−4.00
	Non-small cell lung cancer	*NCI-H23*	−4.50	>−4.00	>−4.00
	Renal cancer	*A-498*	−4.75	−4.43	−4.11
	Breast cancer	*MDA-MB-231/ATTC*	−4.58	>−4.00	>−4.00
		HS-578T	−4.66	−4.28	>−4.00
Ig	Non-small cell lung cancer	*NCI-H23*	−4.58	−4.05	>−4.00
	Colon cancer	*HCT-116*	−4.52	>−4.00	>−4.00
	CNS cancer	*U251*	−4.59	−4.16	>−4.00
	Ovarian cancer	*OVCAR-3*	−4.51	−4.02	>−4.00
	Breast cancer	*MCF-7*	−5.55	>−4.00	>−4.00
		MDA-MB-231/ATTC	−4.63	−4.23	>−4.00
Ij	Leukemia	*CCRF-CEM*	−4.66	>−4.00	>−4.00
		SR	−5.14	−4.70	−4.31
	Non-small cell lung cancer	*HOP-92*	−5.22	−4.62	−4.16
		NCI-H226	−4.58	>−4.00	>−4.00
	CNS cancer	*SF-295*	−4.51	>−4.00	>−4.00
		SNB-75	−5.57	−4.78	>−4.00
		U251	−5.03	−4.20	>−4.00
	Ovarian cancer	*SK-OV-3*	−4.71	−4.09	>−4.00
	Renal cancer	*786-O*	−4.66	>−4.00	>−4.00
	Breast cancer	*MDA-MB-231/ATTC*	−4.80	>−4.00	>−4.00
		HS-578	−4.73	−4.04	>−4.00
Il	Leukemia	*CCRF-CEM*	−6.71	>−4.00	>−4.00
	CNS cancer	*U251*	−4.51	>−4.00	>−4.00

[a] Data of repeat assay.

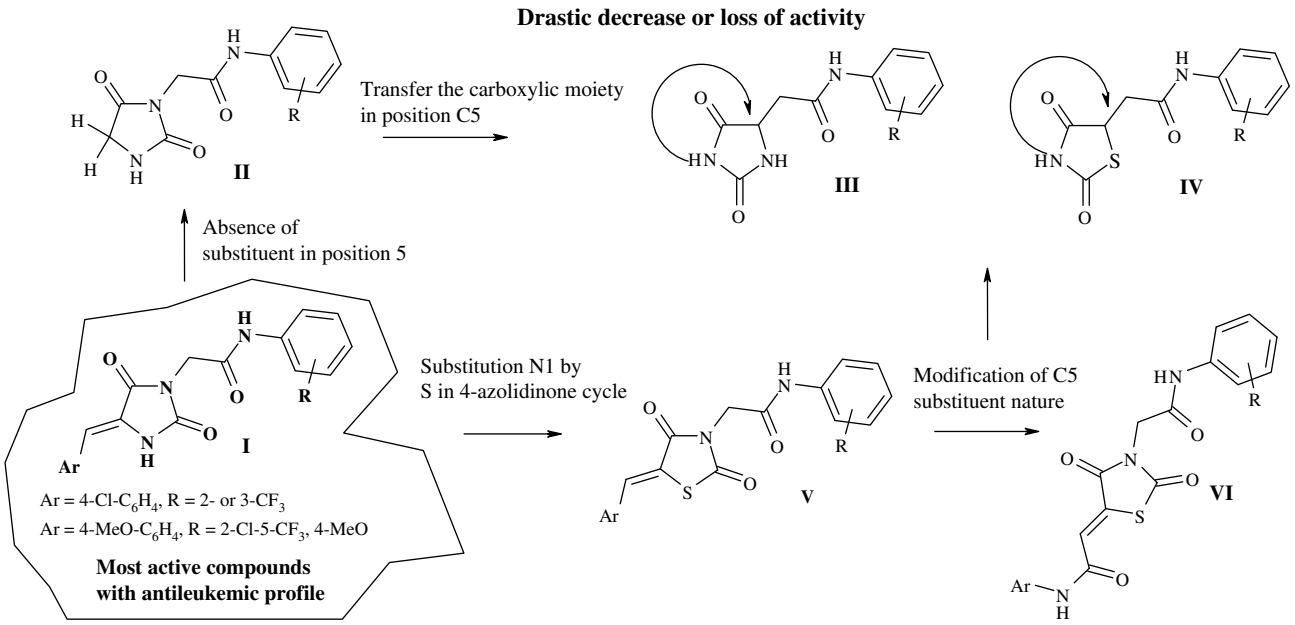


Fig. 3. Directions of chemical modifications of 2,4-azolidinedione derivatives and their influence on anticancer activity.

(SF-268) – concentration 10^{-4} M) or approximately 60 cell lines (concentration 10^{-5} M). The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers. In the screening protocol, each cell line was inoculated and preincubated for 24–48 h on a microtiter plate. Test agents were then added at a single concentration and the culture was incubated for further 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). Results for each test agent were reported as the percent growth of the treated cells when compared to the untreated control cells. The preliminary screening results are shown in Tables 1 and 2.

Compounds that reduced the growth of any of the cell line to approximately 32% or less in the three cell line assay were considered to be active. The compound **Ia** was active towards NCI-H460 and **Ie** – NCI-H460 and MCF7 cell lines and therefore were screened towards about 60 cell lines of above-mentioned types of human cancers at five different concentrations (10^{-4}–10^{-8} M). None of compounds tested at 10^{-5} M was selected for advanced assay. Compounds **Ic**, **Id**, **Ie**, **Ig**, **Ij**, **Il** were tested in five concentration assay without preliminary screening. A 48 h continuous drug exposure protocol was used with a sulforhodamine B (SRB) protein assay to estimate cell viability and growth. Results (Tables 3 and 4) are expressed as log GI_{50}, log TGI, log LC_{50}. (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; LC_{50} – molar concentration of the compound leading to 50% net cell death). Values were calculated for each of these parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value was expressed as more or less than the maximum or minimum concentration tested. Furthermore, mean graph midpoints (MG_MID) were calculated for each of the parameters, giving an average activity parameter over all cell lines for each compound. For the calculation of the MG_MID, insensitive cell lines were included with the highest concentration tested. The dose-dependent 60 cell line assay is summarized in Tables 3 and 4.

The tested compounds showed different strength of anticancer activity – having weak average values of anticancer activity, possesses the significant specific influence on some cancer cell lines. This activity pattern appeared probably due to distinctive molecular mechanisms of action for mentioned substances. Thus compounds **If**, **IIb**, **IVa**, **Ie** and **Ij** specifically restrained the growth of renal cancer lines. UO-31 cell growth suppression, as well as cell death was observed for **If**. Compound **If** and **Id** showed also the specific influence on ovarian cancer. The cells of colon cancer lines are the most sensitive to compound **Ik**. Significant effect of **Id** and **Ij** on CNS cancer cell lines was observed as well.

It is interesting, that compounds **Ia**, **Id**, **Ig** and **Ij** showed prominent antiproliferative effect on breast cancer cell lines, particularly **Ia**, **Id** and **Ij**. The latter were more active in case of MDA-MB-231/ATTC cell line, which is known for its high malignancy and metastatic potential, compared to MCF-7 cell line, effect on which is less significant. In contrast such effects of compound **Ig** were not observed, whereas MCF-7 cell line is more susceptible.

Among tested cancer cell lines the leukemic lines are the most susceptible to 2-(5-arylidene-2,4-dioxoimidazolidin-3-yl)-N-arylacetamides' influence. Thus compounds **Ii**, **Ik** and especially **Ia**, **Ic**, **Id**, **Ij**, **Il** and **Vg** were characterized by the strong and significant effect on leukemia cell lines, whereas compounds **Ic** and **Il** showed distinctive selectivity and didn't inhibit the growth of other cell lines. One should note that compounds **Id** and **Ia** possessed the strongest antimitotic effect.

The SAR study revealed that anticancer activity of compounds **I** was significantly decreased or disappeared after transformation into sulphur-containing analogues or similar systems (**II**–**VI**) (Fig. 3).

These data are suggestive with the high grade of probability that the substitution of sulphur-atom with nitrogen in 2,4-azolidinedione-3-acetic acids derivatives leads to increase of anticancer activity. However, this dependence needs further studies. Nevertheless, we suggest the above-mentioned substitution as the possible route to modelling the substances with anticancer activity. Our data also support the crucial role of the presence and nature of substituent in position 5 of core heterocycles in the anticancer effect realization of 4-azolidinones and benefits of 5-arylidene-substituted series (**I**) compared to other compounds (**III**, **IV**, **VI**).

Most of the compounds contain two or more fluorine and/or chlorine atoms. That could be suggested as possible way of structure optimization for these heterocycles. For example, the substitution of metoxylic groups of **Ig** by Cl atom – **Ia** or the substitution of two metoxylic groups of **Il** by Cl atoms into compound **Id** leads to the significant increase of their activity. Similar increase of activity is observed in case of the inclusion of additional Cl atom into compound **Ig** (compound **Ij**).

3. Conclusion

We developed a new class of potent anticancer agents, namely amides of 5-arylidene-2,4-imidazolidinedione-3-acetic acids, some of which showed selectivity for leukemia cancer cell lines. 2-[5-(4-Chlorobenzylidene)-2,4-dioxo-imidazolidin-3-yl]-N-(2-trifluoromethyl-phenyl)-acetamide (**Ic**) may serve as a useful lead compound in search for powerful and selective antioneoplastic agents.

4. Experimental

4.1. Chemistry

The starting (2,4-dioxo-thiazolidin-5-yl)-acetic acid [37], (2,4-dioxo-thiazolidin-5-ylidene)-acetic acid [43] and (2,5-dioxo-imidazolidin-4-yl)-acetic acid [44] were obtained according to methods described previously, as well as the N-potassium salts and respective amides [37].

Melting points were measured in open capillary tubes on a BŰCHI B-545 melting point apparatus and are uncorrected. The elemental analyses (C, H, N) were performed using the Perkin–Elmer 2400 CHN analyzer and were within ±0.4% of the theoretical values. The ^{1}H NMR spectra were recorded on Varian Gemini 300 MHz in DMSO-d_6 or DMSO-d_6 + CCl_4 mixture using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in ppm units with use of δ scale. LC-MS and EI-MS were obtained on Agilent 1100 and Varian 1200L instruments correspondingly. IR spectra were recorded on a BRUKER-Alfa with ATR nozzle.

4.1.1. General procedure for synthesis of compounds *I, II, V* and *VI*

A mixture 10 mmol of N-potassium salt of appropriative 5-substituted and non-substituted 2,4-imid(thi)azolinediones and 11 mmol N-chloroacetamide derivative, 15 ml of DMF (**I**) or 15 ml of mixture DMF–EtOH (vol. 1:1) (**II**, **V**, **VI**) were added, as well as catalytic amount of potassium iodide and carbonate and refluxed for 4 h. Reaction product was filtered off after cooling and pouring into water, washed by water, ethanol, and diethyl ether. Recrystallized with the mixture of DMF–ethanol (1:2) or acetic acid.

*4.1.1.1. 2-[5-(4-Chloro-benzylidene)-2,4-dioxo-imidazolidin-3-yl]-N-(3-trifluoromethyl-phenyl)-acetamide (**Ia**).* Yield 57%, mp 268–270 °C. IR [cm^{-1}] 3265 (NH), 1767, 1716, 1658 (CO), 1589 (Ar-CH=). ^{1}H NMR [DMSO-d_6] δ: 4.35 (s, 2H), 6.58 (s, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.43 (d, J = 8.0 Hz, 2H), 7.53 (t, J = 8.0 Hz, 1H), 7,67 (d, J = 8.0 Hz, 1H), 7.75 (d, J = 8.0 Hz, 2H), 8.04c (s, 1H), 10.59 (s, 1H) 10.98 (s, 1H). EI-MS (m/z): 423 (M^+), 425 ($M^+ + 2$). Calcd. for $C_{19}H_{13}ClF_3N_3O_3$: C, 53.85; H, 3.09; N, 9.92; Found: C, 54.00; H, 3.00; N, 10.00%.

*4.1.1.2. 2-[5-(4-Fluoro-benzylidene)-2,4-dioxo-imidazolidin-3-yl]-N-(2-trifluoromethyl-phenyl)-acetamide (**Ib**).* Yield 60%, mp 294–296 °C. ^{1}H NMR [DMSO-d_6] δ: 4.35(s, 2H), 6.57 (s, 1H), 7.19 (t, J = 8.0 Hz, 2H), 7.45 (t, J = 8.0 Hz, 1H), 7.52 (d, J = 8.0 Hz, 1H), 7.64–7.72 (m, 4H), 9.90 (s, 1H), 10.88 (s, 1H). EI-MS (m/z): 408 ($M^+ + 1$). Calcd. for $C_{19}H_{13}F_4N_3O_3$: C, 56.03; H, 3.22; N 10.32; Found: C, 56.10; H, 3.30; N, 10.40%.

*4.1.1.3. 2-[5-(4-Chloro-benzylidene)-2,4-dioxo-imidazolidin-3-yl]-N-(2-trifluoromethyl-phenyl)-acetamide (**Ic**).* Yield 56%, mp 298–300 °C. ^{1}H NMR [DMSO-d_6] δ: 4.35 (s, 2H), 6.56 (s, 1H), 7.42 (d, J = 8.0 Hz, 2H), 7.45 (t, J = 8.0 Hz, 1H), 7.52 (d, J = 8.0 Hz, 1H), 7.64–7.69 (m, 3H), 7.71 (d, J = 8.0 Hz, 1H), 9.90 (s, 1H), 10.93 (s, 1H). EI-MS (m/z): 423 (M^+), 425 ($M^+ + 2$). Calcd. for $C_{19}H_{13}ClF_3N_3O_3$: C, 53.85; H, 3.09; N, 9.92; Found: C, 54.00; H, 3.00; N, 10.00%.

*4.1.1.4. 2-[5-(4-Chloro-benzylidene)-2,4-dioxo-imidazolidin-3-yl]-N-(4-chloro-phenyl)-acetamide (**Id**).* Yield 80%, mp 320–322 °C. IR [cm^{-1}] 3324, 3295 (NH), 1779, 1707, 1649 (CO), 1601 (Ar-CH=). ^{1}H NMR [DMSO-d_6] δ: 4.31 (s, 2H), 6.57 (s, 1H), 7.31 (d, J = 8.0 Hz, 2H), 7.42 (d, J = 8.0 Hz, 2H), 7.59 (d, J = 8.0 Hz, 2H), 7.67 (d, J = 8.0 Hz, 2H), 10.38 (s, 1H), 10.96 (s, 1H). EI-MS (m/z): 389 (M^+), 391 ($M^+ + 2$). Calcd. for $C_{18}H_{13}Cl_2N_3O_3$: C, 55.40; H, 3.36; N, 10.77; Found: C, 55.20; H, 3.40; N, 10.85%.

*4.1.1.5. N-(4-Chloro-phenyl)-2-[2,4-dioxo-5-(3-phenyl-allylidene)-imidazolidin-3-yl]-acetamide (**Ie**).* Yield 80%, mp 308–310 °C. IR [cm^{-1}] 3227(NH), 1765, 1714, 1664 (CO), 1594 (Ar-CH=). ^{1}H NMR [DMSO-d_6] δ: 4.27(s, 2H), 6.42 (d, J = 12.0, 1H), 6.95 (m, 1H), 7.28–7.40 (m, 6H), 7.52 (d, J = 8.0 Hz, 2H), 7.59 (d, J = 8.0 Hz, 2H), 10.36 (s, 1H), 11.02 (s, 1H). EI-MS (m/z): 381 (M^+), 383 ($M^+ + 2$). Calcd. for $C_{20}H_{16}ClN_3O_3$: C, 62.91; H, 4.22; N, 11.01; Found: C, 63.00; H, 4.30; N, 11.20%.

*4.1.1.6. 2-(5-Benzylidene-2,4-dioxo-imidazolidin-3-yl)-N-(4-chloro-phenyl)-acetamide (**If**).* Yield 62%, mp 290–292 °C. ^{1}H NMR [DMSO-d_6] δ: 4.32 (s, 2H), 6.59 (s, 1H), 7.30–7.43 (m, 5H), 7.58 (d, J = 8.9 Hz, 2H), 7.66 (d, J = 7.6 Hz, 2H), 10.45 (s, 1H), 10.93 (s, 1H). EI-MS (m/z): 355 (M^+), 357 ($M^+ + 2$). Calcd. for $C_{18}H_{14}ClN_3O_3$: C, 60.77; H, 3.97; N, 11.81; Found: C, 60.85; H, 4.05; N, 12.00%.

*4.1.1.7. 2-[5-(4-Methoxy-benzylidene)-2,4-dioxo-imidazolidin-3-yl]-N-(3-trifluoromethyl-phenyl)-acetamide (**Ig**).* Yield 63%, mp 240–242 °C. ^{1}H NMR [DMSO-d_6] δ: 3.85 (s, 3H), 4.35 (s, 2H), 6.55 (s, 1H), 6.95 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 7.6 Hz, 1H), 7.52 (t, J = 7.6 Hz, 1H), 7.61 (d, J = 8.0 Hz, 2H), 7.76 (d, J = 8.0 Hz, 1H), 8.04 (s, 1H), 10.56 (s, 1H), 10.75 (s, 1H). EI-MS (m/z): 420 ($M^+ + 1$). Calcd. for $C_{20}H_{16}F_3N_3O_4$: C, 57.28; H, 3.85; N, 10.02; Found: C, 57.35; H, 4.00; N, 10.20%.

*4.1.1.8. 2-[5-(4-Methoxy-benzylidene)-2,4-dioxo-imidazolidin-3-yl]-N-thiazol-2-yl-acetamide (**Ih**).* Yield 67%, mp 297–299 °C. ^{1}H NMR [DMSO-d_6] δ: 3.82 (s, 3H), 4.30 (s, 2H), 6.54 (s, 1H), 6.95 (d, J = 8.2 Hz, 2H), 7.12 (d, J = 5.2 Hz, 1H), 7.38 (d, J = 5.2 Hz. 1H), 7.58 (d, J = 8.2 Hz, 2H), 10.35 (s, 1H), 10.74 (s, 1H). EI-MS (m/z): 359 ($M^+ + 1$). Calcd. for $C_{16}H_{14}N_4O_4S$: C, 53.62; H, 3.94; N, 15.63; Found: C, 53.50; H, 4.00; N, 15.50%.

*4.1.1.9. 2-[2,4-Dioxo-5-(3-phenyl-allylidene)-imidazolidin-3-yl]-N-(2-trifluoromethyl-phenyl)-acetamide (**Ii**).* Yield 67%, mp 300–303 °C. ^{1}H NMR [DMSO-d_6] δ: 4.29 (s, 2H), 6.41 (d, J = 11.6 Hz, 1H), 6.99 (d, J = 15.5 Hz, 1H), 7.28–7.53 (m, 8H), 7.66 (t, J = 7.7 Hz, 1H), 7.74 (d, J = 7.8 Hz, 1H), 9.98 (s, 1H), 10.03 (s, 1H). EI-MS (m/z): 416 ($M^+ + 1$). Calcd. for $C_{21}H_{16}F_3N_3O_3$: C, 60.72; H, 3.88; N, 10.12; Found: C, 61.00; H, 4.00; N, 10.05%.

*4.1.1.10. N-(2-Chloro-5-trifluoromethyl-phenyl)-2-[5-(4-methoxy-benzylidene)-2,4-dioxo-imidazolidin-3-yl]-acetamide (**Ij**).* Yield 66%, mp 291–293 °C. ^{1}H NMR [DMSO-d_6] δ: 3.84 (s, 3H), 4.48 (s, 2H), 6.53 (s, 1H), 6.90 (d, J = 8.8 Hz, 2H), 7.33 (d, J = 8.2 Hz, 1H), 7.56 (m, 3H), 8.54 (s, 1H), 9.80 (s, 1H), 10.58 (s, 1H). EI-MS (m/z): 453 (M^+), 455

($M^+ + 2$). Calcd. for $C_{20}H_{15}ClF_3N_3O_4$: C, 52.93; H, 3.33; N, 9.26; Found: C, 53.00; H, 3.50; N, 9.45%.

*4.1.1.11. 2-[5-(4-Dimethylamino-benzylidene)-2,4-dioxo-imidazolidin-3-yl]-N-(2-trifluoromethyl-phenyl)-acetamide (**Ik**).* Yield 68%, mp 297–299 °C. ^{1}H NMR [DMSO-d_6] δ: 2,99 (s, 6H), 4.32 (s, 2H), 6.48 (s, 1H), 6.69 (d, $J = 8.5$ Hz, 2H), 7.39–7.50 (m, 4H), 7.64 (t, $J = 7.6$ Hz, 1H), 7.70 (d, $J = 7.9$ Hz, 1H), 9.79 (s, 1H), 10.49 (s, 1H). EI-MS (m/z): 433 ($M^+ + 1$). Calcd. for $C_{21}H_{19}F_3N_4O_3$: C, 58.33; H, 4.43; N, 12.96; Found: C, 58.50; H, 4.50; N, 13.00%.

*4.1.1.12. 2-[5-(4-Methoxy-benzylidene)-2,4-dioxo-imidazolidin-3-yl] -N-(4-methoxy-phenyl)-acetamide (**Il**).* Yield 81%, mp >230 °C. ^{1}H NMR [DMSO-d_6] δ: 3.74 (s, 3H), 3.82 (s, 3H), 4.26 (s, 2H), 6.53 (s, 1H), 6.83 (d, $J = 8.8$ Hz, 2H), 6.95 (d, $J = 8.8$ Hz, 2H), 7.47 (d, $J = 8.8$ Hz, 2H), 7.60 (d, $J = 8.8$ Hz, 2H), 10.05 (s, 1H), 10.72 (s, 1H). LC-MS, ($M^+ + 1$) *I*% – 383.2 (82.15). Calcd. for $C_{20}H_{19}N_3O_5$: C, 62.99; H, 5.02; N, 11.02; Found: C, 63.10; H, 5.10; N, 10.90%.

*4.1.1.13. 2-(2,4-Dioxo-imidazolidin-3-yl)-N-(3-trifluoromethyl-phenyl) -acetamide (**IIa**).* Yield 78%, mp 208–210 °C. IR [cm^{-1}] 3309, 3253 (NH), 1775, 1714, 1678 (CO), ^{1}H NMR [DMSO-d_6] δ: 4.00 (s, 2H), 4.19 (s, 2H), 7.41 (d, $J = 7.7$ Hz, 1H), 7.56 (t, $J = 8.0$ Hz, 2H), 7.71 (d, $J = 8.4$ Hz, 1H), 8.04 (s, 1H), 8.20 (s, 1H), 10.59 (s, 1H). EI-MS (m/z): 301 (M^+). Calcd. for $C_{12}H_{10}F_3N_3O_3$: C, 47.85; H, 3.35; N, 13.95; Found: C, 48.00; H, 3.45; N, 14.05%.

*4.1.1.14. 2-(2,4-Dioxo-imidazolidin-3-yl)-N-(4-sulfamoyl-phenyl)-acetamide (**IIb**).* Yield 82%, mp 198–200 °C. ^{1}H NMR [DMSO-d_6] δ: 4.00 (s, 2H), 4.20 (s, 2H), 7.26 (s, 2H), 7.68 (d, $J = 8.5$ Hz, 2H), 7.76 (d, $J = 8.5$ Hz, 2H), 8.19 (s, 1H), 10.58 (s, 1H). EI-MS (m/z): 312 (M^+). Calcd. for $C_{11}H_{12}N_4O_5S$: C, 42.31; H, 3.87; N, 17.94; Found: C, 42.50; H, 4.00; N, 18.05%.

*4.1.1.15. 2-[5-(4-Chloro-benzylidene)-2,4-dioxo-thiazolidin-3-yl]-N-(3-trifluoromethyl-phenyl)-acetamide (**Va**).* Yield 83%, mp 237–238 °C. ^{1}H NMR [DMSO-d_6] δ: 4.53 (s, 1H), 7.34 (d, $J = 8.0$ Hz, 1H), 7.51 (t, $J = 8.0$ Hz, 1H), 7.54 (d, $J = 8.4$ Hz, 2H), 7.64 (d, $J = 8.4$ Hz, 2H), 7.75 (d, $J = 8.0$ Hz, 1H), 7.96 (s, 1H), 8.0 (s, 1H), 10.63 (s, 1H). EI-MS (m/z): 440 (M^+), 442 ($M^+ + 2$). Calcd. for $C_{19}H_{12}ClF_3N_2O_3S$: C, 51.77; H, 2.74; N, 6.35; Found: C, 52.00; H, 2.80; N, 6.45%.

*4.1.1.16. 2-[5-(4-Chloro-benzylidene)-2,4-dioxo-thiazolidin-3-yl]-N-(2-trifluoromethyl-phenyl)-acetamide (**Vb**).* Yield 79%, mp 256–258 °C. ^{1}H NMR [DMSO-d_6] δ: 4.52 (s, 2H), 7.42–7.50 (m, 2H), 7.51–7.55 (m, 8H), 7.99 (s, 1H), 10.12 (s, 1H). EI-MS (m/z): 440 (M^+), 442 ($M^+ + 2$). Calcd. for $C_{19}H_{12}ClF_3N_2O_3S$: C, 51.77; H, 2.74; N, 6.35; Found: C, 51.90; H, 2.85; N, 6.20%.

*4.1.1.17. 2-[5-(4-Chloro-benzylidene)-2,4-dioxo-thiazolidin-3-yl]-N-(4-chloro-phenyl)-acetamide (**Vc**).* Yield 88%, mp >260 °C. IR [cm^{-1}] 3257 (NH), 1748, 1688, 1669 (CO), 1605 (Ar-CH=). ^{1}H NMR [DMSO-d_6] δ: 4.96 (s, 2H), 7.28 (d, $J = 8.8$ Hz, 2H), 7.54 (d, $J = 8.8$ Hz, 2H), 7.58 (d, $J = 8.8$ Hz, 2H), 7.63 (d, $J = 8.8$ Hz, 2H), 7.95 (s, 1H), 10.41 (s, 1H). EI-MS (m/z): 408 ($M^+ + 1$). Calcd. for $C_{18}H_{12}Cl_2N_2O_3S$: C, 53.08; H, 2.97; N, 6.88; Found: C, 52.90; H, 3.05; N, 7.00%.

*4.1.1.18. N-(4-Chloro-phenyl)-2-[2,4-dioxo-5-(3-phenyl-allylidene)-thiazolidin-3-yl]-acetamide (**Vd**).* Yield 62%, mp 275–276 °C. IR [cm^{-1}] 3319 (NH), 1741, 1680 (CO), 1583 (Ar-CH=). ^{1}H NMR [DMSO-d_6] δ: 4.42(s, 2H), 7.04 (dd, $J = 11.3$ Hz, $J = 15.2$ Hz, 1H,), 7.30–7.47 (m, 6H), 7.49–7.61 (m, 2H), 7.62–7.76 (m, 3H), 10.52 (s, 1H). EI-MS (m/z): 398 (M^+) 400 ($M^+ + 2$). Calcd. for $C_{20}H_{15}ClN_2O_3S$: C, 60.23; H, 3.79; N, 7.02; Found: C, 60.30; H, 3.90; N, 7.20%.

*4.1.1.19. 2-[5-(4-Methoxy-benzylidene)-2,4-dioxo-thiazolidin-3-yl]-N-thiazol-2-yl-acetamide (**Ve**).* Yield 88%, mp 289–290 °C. ^{1}H NMR [DMSO-d_6] δ: 3.88 (s, 3H), 4.60 (s, 2H), 6.99 (d, $J = 5.2$ Hz, 1H), 7.08 (d, $J = 8.8$ Hz, 2H), 7.38 (d, $J = 5.2$ Hz, 1H), 7.53 (d, $J = 8.8$ Hz, 2H), 7.88 (s, 1H), 12.30 (s, 1H). EI-MS (m/z): 375 (M^+). Calcd. for $C_{16}H_{13}N_3O_4S_2$: C, 51.19; H, 3.49; N, 11.19; Found: C, 51.00; H, 3.55; N, 11.30%.

*4.1.1.20. 2-[2,4-Dioxo-5-(3-phenyl-allylidene)-thiazolidin-3-yl]-N-(2-trifluoromethyl-phenyl)-acetamid (**Vf**).* Yield 81%, mp 306–308 °C. ^{1}H NMR [DMSO-d_6] δ: 4.48 (s, 2H), 7.02 (dd, $J = 11.3$ Hz, $J = 15.1$ Hz, 1H), 7.23–7.52 (m, 6H), 7.56–7.78 (m, 2H), 10.12 (s, 1H). EI-MS (m/z): 433 ($M^+ + 1$). Calcd. for $C_{21}H_{15}F_3N_2O_3S$: C, 58.33; H, 3.50; N, 6.48; Found: C, 58.20; H, 3.70; N, 6.30%.

*4.1.1.21. 2-[5-(4-Dimethylamino-benzylidene)-2,4-dioxo-thiazolidin-3-yl]-N-(2-trifluoromethyl-phenyl)-acetamide (**Vg**).* Yield 79%, mp 296–298 °C. ^{1}H NMR [DMSO-d_6] δ: 3.05 (s, 6H), 4.49 (s, 1H), 6.80 (d, $J = 8.9$ Hz, 2H), 7.44 (m, 3H), 7.51 (d, $J = 8.2$ Hz, 1H), 7.64 (d, $J = 8.2$ Hz, 1H), 7.72 (d, $J = 8.2$ Hz, 1H), 7.80 (s, 1H), 9.94 (s, 1H). EI-MS (m/z): 450 ($M^+ + 1$). Calcd. for $C_{21}H_{18}F_3N_3O_3S$: C, 56.12; H, 4.04; N, 9.35; Found: C, 56.00; H, 3.90; N, 9.50%.

*4.1.1.22. N-(4-Chloro-phenyl)-2-{2,4-dioxo-3-[(3-trifluoromethyl-phenylcarbamoyl)-methyl]-thiazolidin-5-ylidene}-acetamide (**VIa**).* Yield 56%, mp 284–286 °C. IR [cm^{-1}] 3339 (NH), 1739, 1687, 1658 (CO), 1601. ^{1}H NMR [DMSO-d_6] δ: 4.55 (s, 2H), 7.40 (s, 1H), 7.42 (d, $J = 8.0$ Hz, 1H) 7.44 (d, $J = 8.8$ Hz, 2H), 7.72 (d, $J = 8.8$ Hz, 2H), 7.59 (t, $J = 8.0$ Hz, 1H), 7.75 (d, $J = 8.0$ Hz, 1H), 8.05 (s, 1H), 10.75 (s, 1H), 11.05 (s, 1H). Calcd. for $C_{20}H_{13}ClF_3N_3O_4S$: C, 49.65; H, 2.71; N, 8.68; Found: C, 49.50; H, 2.80; N, 8.50%.

*4.1.1.23. 2-{2,4-Dioxo-3-[(2-trifluoromethyl-phenylcarbamoyl)-methyl]-thiazolidin-5-ylidene}-N-(4-fluoro-phenyl)-acetamide (**VIb**).* Yield 48%, mp 244–246 °C. ^{1}H NMR [DMSO-d_6] δ: 4.51(s, 2H), 7.21 (t, $J = 9.0$ Hz, 2H), 7.38 (s, 1H), 7.48 (m, 2H), 7.64–7.78 (m, 4H), 10.07 (s, 1H), 10.94 (s, 1H). Calcd. for $C_{20}H_{13}F_4N_3O_4S$: C, 51.40; H, 2.80; N, 8.99; Found: C, 51.50; H, 3.00; N, 9.10%.

*4.1.1.24. N-(4-Chloro-phenyl)-2-{2,4-dioxo-3-[(2-trifluoromethyl-phenylcarbamoyl)-methyl]-thiazolidin-5-ylidene}-acetamide (**VIc**).* Yield 52%, mp 260–262 °C. IR [cm^{-1}] 3366 (NH), 1714, 1685, 1658 (CO), 1599. ^{1}H NMR [DMSO-d_6] δ: 4.51(s, 2H), 7.35 (s, 1H), 7.38 (d, $J = 8.0$ Hz, 2H), 7.46 (t, $J = 8.0$ Hz, 1H), 7.50 (d, $J = 8.0$ Hz, 1H), 7.66 (t, $J = 8.0$ Hz, 1H) 7.69 (d, $J = 8.0$ Hz, 2H), 7.72 (d, $J = 8.0$ Hz, 1H), 10.00 (s, 1H), 10.95 (s, 1H). Calcd. for $C_{20}H_{13}ClF_3N_3O_4S$: C, 49.65; H, 2.71; N, 8.68; Found: C, 49.50; H, 2.95; N, 8.50%.

*4.1.1.25. N-(4-Chloro-phenyl)-2-{3-[(4-chloro-phenylcarbamoyl)-methyl]-2,4-dioxo-thiazolidin-5-ylidene}-acetamide (**VId**).* Yield 46%, mp 290–292 °C. IR [cm^{-1}] 3344 (NH), 1741, 1685, 1658 (CO), 1599. ^{1}H NMR [DMSO-d_6] δ: 4.47(s, 2H), 7.27 (d, $J = 8.0$ Hz, 2H), 7.31(d, $J = 8.0$ Hz, 2H), 7.40 (s, 1H), 7.57 (d, $J = 8.0$ Hz, 2H), 7.69 (d, $J = 8.0$ Hz, 2H), 10.40 (s, 1H), 10.88 (s, 1H). Calcd. for $C_{19}H_{13}Cl_2N_3O_4S$: C, 50.68; H, 2.91; N, 9.33; Found: C, 50.50; H, 3.00; N, 9.10%.

*4.1.2. General procedure for synthesis of compounds **III** and **IV***

A mixture of 5 mmol of appropriate acid and 1.2 g of thionyl chloride in 3 ml of dioxane was heated under reflux for 0.5 h, cooled, and treated with 10 ml of hexane. The precipitate was separated by filtration and used without further purification. To a solution of 10 mmol of the corresponding amine and 1 ml of triethylamine in 10 ml of anhydrous dioxane was added the solution of 10 mmol of respective acid chloride in 10 ml of the same solvent. The mixture was heated for 10 min at 100 °C, cooled, and

diluted with 100 ml of water. The precipitate was separated by filtration and recrystallized with an appropriate solvent.

*4.1.2.1. 2-(2,4-Dioxo-imidazolidin-5-yl)-N-(4-sulfamoyl-phenyl)-acetamide (**IIIa**).* Yield 78%, mp 208–210 °C. ^{1}H NMR [DMSO-d_6] δ: 2.60 (dd, $J = 16.0$ Hz, $J = 8.4$ Hz, 1H), 2.77 (dd, $J = 16.0$ Hz, $J = 4.0$ Hz, 1H), 4.31 (dd, $J = 8.4$ Hz, $J = 4.0$ Hz, 1H), 7.26 (s, 2H), 7.72 (d, $J = 9.0$ Hz, 2H), 7.76 (d, $J = 9.0$ Hz, 2H), 7.93 (s, 1H), 10.39 (s, 1H), 10.64 (s, 1H). EI-MS (m/z): 312(M^+). Calcd. for $C_{11}H_{12}N_4O_5S$: C, 42.31; H, 3.87; N, 17.94; Found: C, 42.50; H, 3.95; N, 18.05%.

*4.1.2.2. N-(4-Chloro-phenyl)-2-(2,4-dioxo-imidazolidin-5-yl)-acetamide (**IIIb**).* Yield 61%, mp 212–214 °C. ^{1}H NMR [DMSO-d_6] δ: 2.60 (dd, $J = 16.0$ Hz, $J = 8.0$ Hz, 1H), 2.77 (dd, $J = 16.0$ Hz, $J = 4.0$ Hz, 1H), 4.25 (dd, $J = 8.0$ Hz, $J = 4.0$ Hz, 1H), 7.23 (d, $J = 8.8$ Hz, 2H) 7.57 (d, $J = 8.8$ Hz, 2H) 7.82 (s, 1H), 10.06 (s, 1H), 10.54 (s, 1H). EI-MS (m/z): 267 (M^+) 269 ($M^+ + 2$). Calcd. for $C_{11}H_{10}ClN_3O_3$: C, 49.36; H, 3.77; N, 15.70; Found: C, 49.10; H, 3.65; N, 15.50%.

*4.1.2.3. 2-(2,4-Dioxo-imidazolidin-5-yl)-N-thiazol-2-yl-acetamide (**IIIc**).* Yield 60%, mp 290–292 °C. IR [cm^{-1}] 3185 (NH), 1767, 1681 (CO), 1573. ^{1}H NMR [DMSO-d_6] δ: 2.77 (dd, $J = 15.1$, Hz, $J = 8.2$ Hz, 1H), 2.89 (dd, 1H, $J = 15.1$ Hz, $J = 3.9$ Hz, 1H), 4.31 (m, 1H), 7.13 (d, $J = 5.1$ Hz, 1H) 7.40 (d, $J = 5.1$ Hz, 1H) 7.85 (s, 1H) 10.61 (s, 1H), 12.16 (s, 1H). EI-MS (m/z): 241 ($M^+ + 1$). Calcd. for $C_8H_8N_4O_3S$: C, 40.00; H, 3.36; N, 23.32; Found: C, 39.80; H, 3.50; N, 23.50%.

*4.1.2.4. 2-(2,4-Dioxo-thiazolidin-5-yl)-N-(4-sulfamoyl-phenyl)-acetamide (**IVa**).* Yield 64%, mp 264–266 °C. ^{1}H NMR [DMSO-d_6] δ: 2.98 (m, 1H), 3.27 (dd, 1H, $J = 15.8$ Hz, $J = 4.2$ Hz), 4.52 (dd, 1H, $J = 10.0$ Hz, $J = 4.2$ Hz), 6.98 (s, 2H), 7.65 (d, $J = 8.6$ Hz, 2H), 7.75 (d, $J = 8.6$ Hz, 2H), 10.30 (s, 1H), 11.86 (s, 1H). Calcd. for $C_{11}H_{11}N_3O_5S_2$: C, 40.12; H, 3.37; N, 12.76; Found: C, 40.00; H, 3.50; N, 13.00%.

*4.1.2.5. 2-(2,4-Dioxo-thiazolidin-5-yl)-N-thiazol-2-yl-acetamide (**IVb**).* Yield 58%, mp 242–244 °C. ^{1}H NMR [DMSO-d_6] δ: 3.05 (dd, $J = 17.6$ Hz, $J = 10.3$ Hz, 1H,), 3.30 (dd, $J = 17.6$ Hz, $J = 4.4$ Hz, 1H), 4.55 (m, 1H), 6.95 (d, $J = 5.4$ Hz, 1H), 7.32 (d, $J = 5.4$ Hz, 1H), 11.85 (s, 1H), 12.25 (s, 1H). Calcd. for $C_8H_7N_3O_3S_2$: C, 37.35; H, 2.74; N, 16.33; Found: C, 37.50; H, 2.90; N, 16.50%.

4.2. Cytotoxic activity against malignant human tumor cells

Primary anticancer assay was performed at human tumor cell lines panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda [39–41]. Tested compounds were added to the culture at a single concentration (10^{-3} or 10^{-5} M) and the cultures were incubated for 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). Results for each tested compound were reported as the percent of growth of the treated cells when compared to the untreated control cells. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents. The cytotoxic and/or growth inhibitory effects of the most active selected compounds were tested *in vitro* against the full panel of about 60 human tumor cell lines at 10-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. A 48-h continuous drug exposure protocol was followed and an SRB protein assay was used to estimate cell viability or growth. Using the seven absorbance measurements [time zero, (T_z), control growth in the absence of drug, (C), and test growth in the presence of drug at the five concentration levels (T_i)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

$[(T_i - T_z)/(C - T_z)] \times 100$ for concentrations for which $T_i \geq T_z$,

$[(T_i - T_z)/T_z] \times 100$ for concentrations for which $T_i < T_z$

Three dose response parameters were calculated for each compound. Growth inhibition of 50% (GI50) was calculated from $[(T_i - T_z)/(C - T_z)] \times 100 = 50$, which is the drug 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. The drug concentration resulting in total growth inhibition (TGI) was calculated from $T_i = T_z$. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from $[(T_i - T_z)/T_z] \times 100 = -50$. Values were calculated for each of these three parameters if the level of activity is reached; however, if the effect 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 log GI_{50}, log TGI, log LC_{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.

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