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Original article

Synthesis and anticancer activity evaluation of 4-thiazolidinones containing benzothiazole moiety

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ABSTRACT

Antitumor screening of several novel 4-thiazolidinones with benzothiazole moiety has been performed. Reactions of (benzothiazole-2-yl)hydrazine with trithiocarbonyl diglycolic acid or 6-methyl-2-amino-benzothiazole with 2-carbethoxymethylthio-2-thiazoline-4-one have yielded starting 3- (**1**) or 2-substituted (**11**) 4-thiazolidinones which have been subsequently utilized in a Knoevenagel condensation for obtaining a series of 5-arylidene derivatives **2**–**10**, **12**–**16**. Compound **11** has been obtained alternatively by a counter synthesis method based on the reaction of 2-chloro-N-(6-methylbenzothiazol-2-yl)-acetamide and ammonium thiocyanate. The structures of compounds have been determined by ^{1}H, ^{13}C NMR, IR and X-ray analysis. *In vitro* anticancer activity of the synthesized compounds was tested by the National Cancer Institute and two (**6**, **16**) of them has revealed the anticancer activity on leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers cell lines. Among tested compounds, 2-{2-[3-(benzothiazol-2-ylamino)-4-oxo-2-thioxothiazolidin-5-ylidenemethyl]-4-chlorophenoxy}-N-(4-methoxyphenyl)-acetamide (**6**) was found to be the most active candidate with average $logGI_{50}$ and logTGI values −5.38 and −4.45 respectively.

1. Introduction

4-Thiazolidinone ring system is a core structure in various synthetic compounds displaying broad spectrum of biological activities [1], including an anticancer effect [2–5]. Mechanisms of 4-thiazolidinones and related heterocycles antitumor activity may be associated with the affinity to anticancer biotargets, such as phosphatase of a regenerating liver (PRL-3) [6,7], nonmembrane protein tyrosine phosphatase (SHP-2) [8], JNK-stimulating phosphatase-1 (JSP-1) [9], tumor necrosis factor TNFα [10], anti-apoptotic biocomplex Bcl-X_L-BH3 [11], integrin $\alpha_v\beta_3$ [12], etc. Necroptosis inhibitors have been recently identified among 4-thiazolidinones [13]. On the other hand benzothiazole ring belongs to the privilleged scaffolds in modern medicinal chemistry [14] particularly in discovering of new anticancer agents. Various benzothiazole derivatives were proposed as inhibitors of fatty acid amide hydrolase (FAAH) [15], Raf kinase (Raf-1) [16] and B-cell lymphoma protein BCL-2 [17]. 5-Arylidene derivatives were previously shown as the most active group of compounds with the anticancer activity among large pull of 4-azolidone derivatives and analogs [18]. During the studies presented in this article we have found that attachment of benzothiazole moiety to 5-arylidene-thiazolidinone scaffold allowed as gaining of 1 log of activity (at GI_{50} level) in comparison to 2/3-unsubstituted analogous compound samples. Consequently, the combination of 4-thiazolidinone template with benzothiazole moiety in one molecule can be considered as promising approach in drug-like molecules design (Fig. 1) which has already been partially confirmed by the discovery of MKT 077 [19] reported as a registered antitumor agent. In this spirit, herein we describe the synthesis and anticancer activity of new 4-thiazolidinones with benzothiazole moiety.

2. Results and discussion

2.1. Chemistry

The general synthetic pathways of targeted benzothiazole substituted 4-thiazolidinones are depicted in Schemes 1 and 3

We synthesized 3-(benzothiazol-2-ylamino)-2-thioxo-4-thiazolidone (**1**) from (benzothiazole-2-yl)hydrazine and trithiocarbonyl

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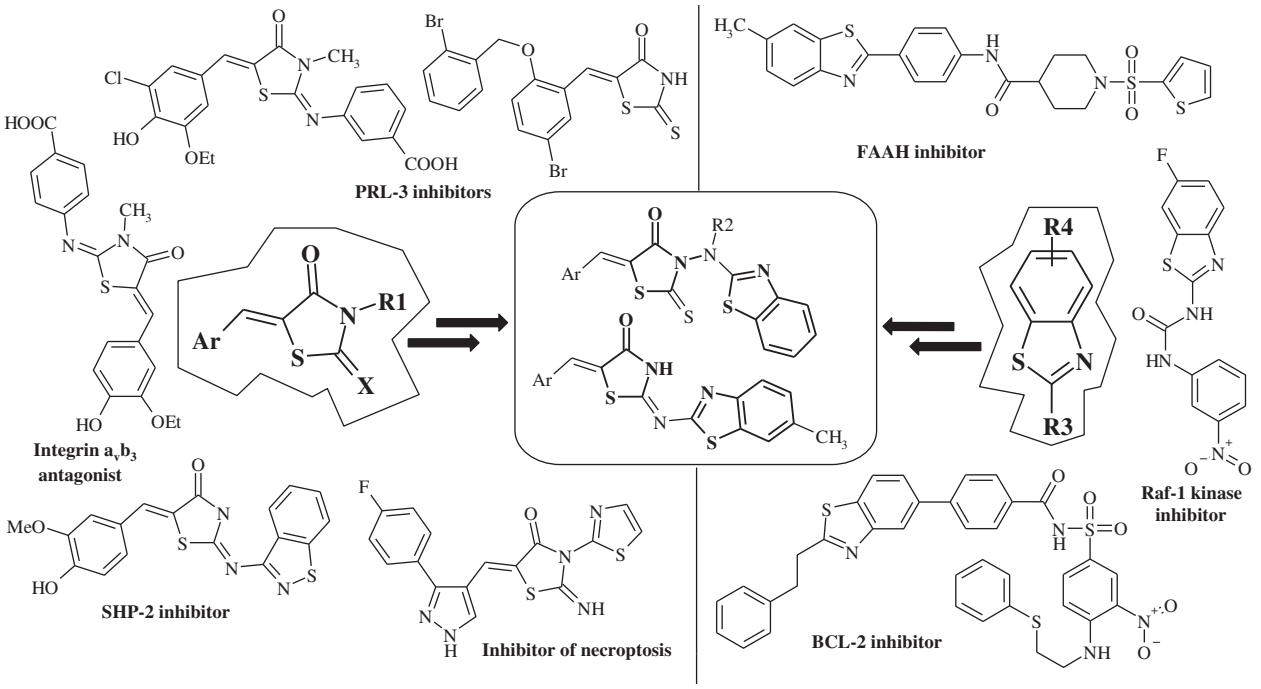
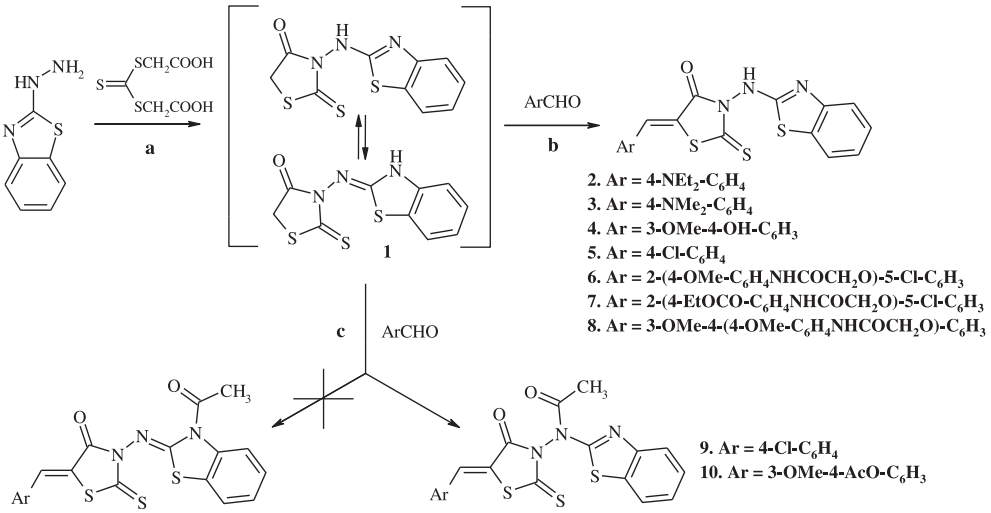


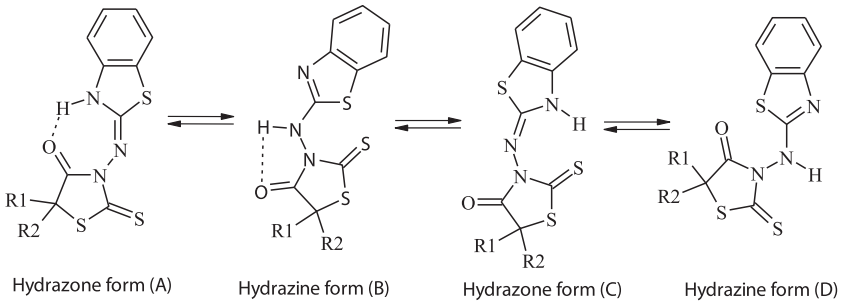
Fig. 1. Structures of anticancer 4-thiazolidinones and benzothiazoles and background for target compounds synthesis.

diglycolic acid [20] in refluxing ethanol with the yield of 78%. The synthesized methylene active derivative **1** was readily reacted with aromatic aldehydes to produce 5-arylidene derivatives **2**–**8**, using a Knoevenagel condensation procedure (medium – acetic acid, catalyst – fused sodium acetate) [1,3,21]. The acetylation of exocyclic nitrogen was observed (**9**–**10**) following acetic anhydride addition to reactive mixture. This fact clearly proved that compound **1** had reacted in hydrazine tautomeric form (Scheme 1), although the characteristic feature of the mentioned substance and its 5-arylidene derivatives **2**–**8** is hydrazone-hydrazine tautomerism (Scheme 2). Additionally, both tautomer forms may exist as a mixture of two stereoisomers (A,C and B,D), which are particularly stabilized by the formation of intramolecular hydrogen bonds.

The prototropic tautomerism and stereoisomerism among this class of compounds was confirmed by spectral data, both in solution and in the solid state. Spectroscopic studies revealed characteristic multiplication of signals in IR, ^{1}H and ^{13}C NMR spectra, observed for some compounds and connected with the co-existence of different tautomeric forms. Thus, in ^{1}H NMR spectra of compounds **1**–**8** NH proton appears as two singlets at ~11.60 ppm and ~12.45 ppm and benzothiazole moiety forms subspectrum of multiplets at ~7.05–7.55 ppm. A *Z*-configuration of the exocyclic C=C bond in the 5-arylidene derivatives **2**–**10** was confirmed by the signal of a methine proton, which resonated at higher chemical shift (~7.80 ppm) as a broad singlet [22,25]. In the solid state, the existence of compounds **1**–**8** in tautomeric



Scheme 1. Synthesis of 3-(benzothiazol-2-ylamino)-2-thioxo-4-thiazolidinones. Reagents, conditions and yields: (a) EtOH, reflux 5h, 78%; (b) AcONa, AcOH, reflux 2h, 59–78%; (c) AcONa, AcOH, Ac_2O, reflux 2h, 71–74%.

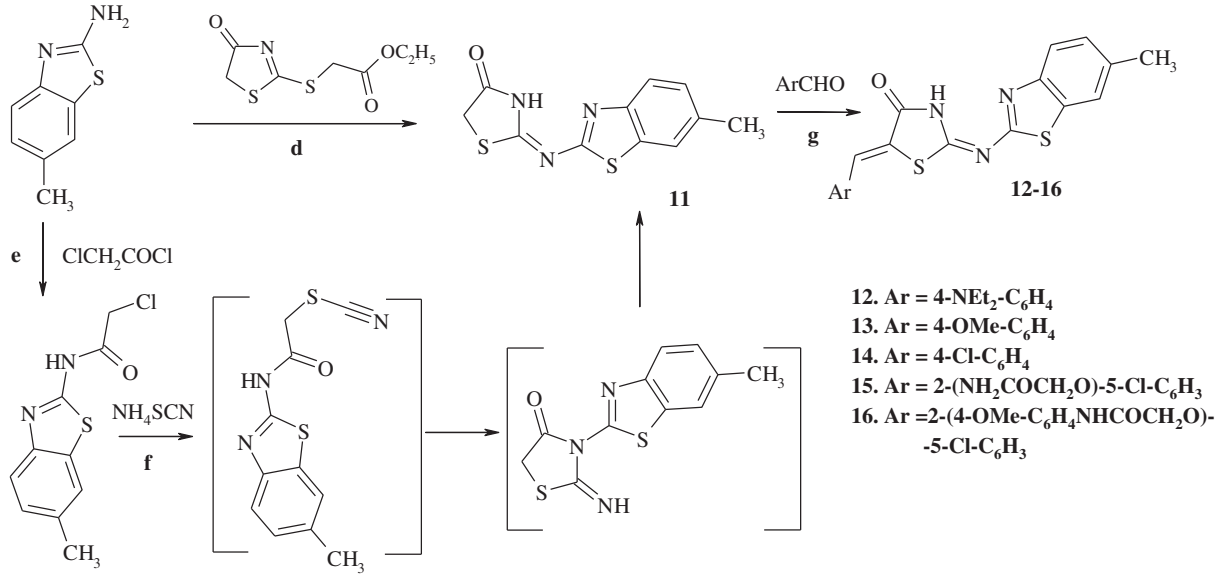


Scheme 2. Hydrazino-hydrazone tautomerism of **2**–**8**.

forms was confirmed by a dublicated band of the C=O group in the 1680–1720 region.

Structural features of synthesized N-benzothiazol-2-yl-N-[5-(4-chlorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]-acetamide (**9**) were confirmed also by X-ray crystallographic analysis. Compound **9** has been crystallized in the space group *P*-1 with two independent molecules in the asymmetric unit, denoted *A* and *B* (Fig. 2), which differed significantly in conformation (weighted r.m.s. = 3.3077 Å). The conformational differences have shown the superimposition of the molecules *A* and *B* obtained by the least-squares fitting of the thiazolidine rings. These dissimilarities concern more the angular arrangement of the benzothiazolylacetamide fragment, less the positioning of the p-chlorobenzylidene residue. The first fragment of the molecule is approximately planar [r.m.s. for the atoms N7A – N19A: 0.0362 Å, for the atoms N7B – N19B: 0.0368 Å] and is perpendicular or almost perpendicular to the plane of the thiazolidine ring [dihedral angle for the molecule *A*: 89.89(3)°, for the molecule *B*: 86.59(4)°]. The acetamide and benzothiazole fragments in the molecules *A* and *B* are located on the opposite sites as a result of the rotation of the whole benzothiazolylacetamide residue around the N3–N7 bond by nearly 180°. This arrangement is confirmed by the torsional angles C2A–N3A–N7A–C8A and C2B–N3B–N7B–C8B, as well as C2A–N3A–N7A–C11A and C2B–N3B–N7B–C11B which take similar values but of the opposite signs [89.86(18) and −90.73 (19)°, as well as −92.61(17) and 90.75(18)°, respectively]. The p-chlorobenzylidene group assumes in the both molecules a *cis*-configuration with respect to the S1–C5 bond. The torsion angles S1–C5–C21–C22 take the values of 0.7(3) and 2.3(3)° for the molecules *A* and *B*, respectively. The dihedral angles between the approximately planar chlorobenzylidene residue and the plane of the thiazolidine ring are 35.30(8)° in the molecule *A* and 19.32(6)° in the molecule *B* due to the rotation around the C21–C22 bond.

In the both molecules the double C5=C21 bond is conjugated with the p-chlorophenyl system and carbonyl group C4=O20. It is interesting to note that the p-Cl–C_6H_5–C21=C5 atom system is folded differently in the molecules *A* and *B* [r.m.s.: 0.1405 and 0.1046 Å, respectively] which is connected with a non-identical inclination of the C5 atom out [-0.571(2) Å (molecule *A*) and −0.400 (2) Å (molecule *B*)] of the plane defined by the remaining atoms of this residue. Despite this, the interatomic distances C21–C22 (Fig. 2) have similar values [1.457(2) Å and 1.450(2) Å in the molecules *A* and *B*] and are corresponded to the literature length of the single bond in (p-Cl–)C_6H_5–C(=C) [1.459(4) Å] [23]. The other conjugated system made of O20=C4–C5=C21 is approximately planar [r.m.s. deviation: 0.0204 and 0.0124 Å in molecule *A* and *B*, respectively]. The bond lengths C4–C5 in the molecules *A* and *B* are 1.469(2) Å and 1.473(2) Å, respectively. They are comparable with the value of the single bond length of (C=)C–C(=O) [1.465(1) Å] [24].



Scheme 3. Synthesis of 2-(6-methylbenzothiazol-2-ylimino)-4-thiazolidinones. Reagents, conditions and yields: (d) EtOH, reflux 2h, 74%; (e) dioxane, rt 2h; (f) acetone, reflux 5h, 59%; (g) AcONa, AcOH, reflux 3h, 68–82%.

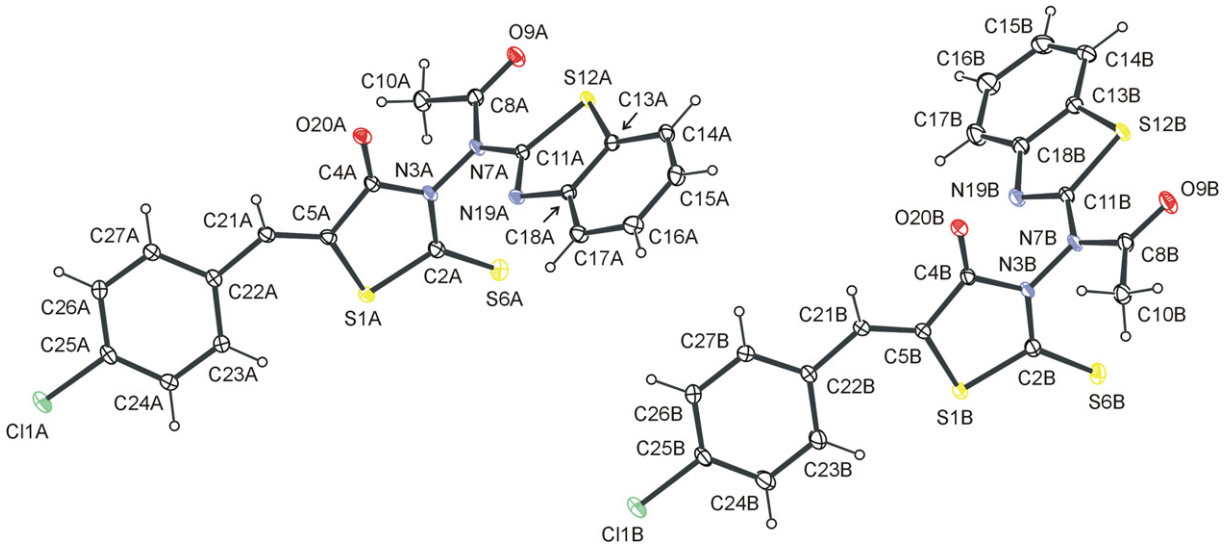


Fig. 2. The structure of two symmetry-independent molecules of **9**, showing the atomic labelling scheme. Displacement ellipsoids are drawn at the 50% probability level and H atoms are depicted as small spheres of an arbitrary radius.

The molecules of compound (**9**) contain two tertiary amide groups connected by the N3–N7 bond. The interatomic distances C4–N3 [1.402(2) Å in the molecule *A* and 1.408(2) Å in the molecule *B*] and C8–N7 [1.4048(19) Å in the molecule *A* and 1.402 (2) Å in the molecule *B*] take similar values and are longer than the standard length of the C–N bond in the tertiary amide group with a $C_{sp}3$–N(–$C_{sp}3$)–C=O atom system, [1.346(5) Å] [24], by about 10–12σ.

The interatomic distances C11–N19 in the benzothiazole system take the values of 1.2912(19) in the molecule *A* and 1.290(2) Å in the molecule *B* and confirm the presence of the double bond between these atoms.

In the crystal lattice (Fig. 3a), molecules *A* and *B* are joined through the three-centre hydrogen bonds C10A–H10A···O20B, C14B^{ii}–H14B^{ii}···O20B [(ii) *1-x,1-y,1-z*;, Table 1] and C14A–H14A···O20A^{i}, C26B–H26B···O20A^{i} [(i) *-x,-y,-z*] into the chains growing in the direction [−111]. The neighbouring chains of molecules are linked through the hydrogen bonds C17B–H17B···Cl1B^{iii} [(iii) *1-x,-y,-z*] into layers parallel to the plane (0–11). The thickness of the layer is close to about 9.4 Å (Fig. 3b).

In the crystal of compound **9**, the rule of the close packing of molecules is not fully satisfied. Total potential solvent accessible voids are 46.3 Å^3, which makes 2.4% of the unit cell volume.

Aiming at the detailed elaboration of the structure–activity relationship, particularly influence of benzothiazole moiety position on 4-thiazolidinones anticancer activity, 5-arylidene-2-(6-methylbenzothiazol-2-ylimino)-4-thiazolidinones (**12**–**16**) were synthesized (Scheme 2). Reaction of 6-methyl-2-aminobenzothiazole with 2-carbethoxymethylthio-2-thiazoline-4-one [3,21] afforded in the excellent yield and purity of the starting 2-substituted 4-thiazolidinone (**11**). The compound **11** has been obtained alternatively by a known counter synthesis method [8,25,26] based on the reaction of 6-methyl-2-aminobenzothiazole with chloroacetyl chloride and following cyclization of 2-chloro-N-(6-methylbenzothiazol-2-yl) acetamide with ammonium thiocyanate in refluxing acetone (Scheme 3). 2-(6-Methylbenzothiazol-2-ylimino)-4-thiazolidinone **11** was used in the Knoevenagel condensation in order to obtain a series of 5-arylidene derivatives **12**–**16** (Scheme 3). In ^{1}H NMR spectra the chemical shift of the methylidene group of 5-arylidene derivatives **12**–**16**, similarly to compounds **2**–**10**, was insignificantly displaced in the weak magnetic field, δ 7.76–7.89 ppm, which clearly indicated that only *Z*-isomers were obtained [22,25] in the Knoevenagel reaction.

The background for the 5-arylidene derivatives synthesis in all cases (compounds **2**–**10**, **12**–**16**) was based on a previously established postulate, that a benzylidene moiety at the 5-position of the 4-thiazolidinone is necessary for the biological activity [1,8,25,26] including anticancer effect [2,3,5,18].

The characterization data of the synthesized novel benzothiazole substituted thiazolidones are presented in experimental part. Analytical and spectral data (^{1}H NMR, ^{13}C NMR, IR) confirmed the structure of synthesized compounds.

2.2. Evaluation of anticancer activity in vitro

The synthesized benzothiazole substituted 4-thiazolidinones (**2, 4, 6–8, 12–16**) were evaluated at the single concentration of 10^{-5} M towards panel of the approximately sixty cancer cell lines. The human tumor cell lines were derived from the nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers. Primary anticancer assays were performed according to the US NCI protocol, which was described elsewhere [27–31]. The compounds were added at single concentration and the cell culture was incubated for 48 h. End-point determinations were made with a protein binding dye, sulforhodamine B (SRB). The results for each compound are reported as the percent growth (GP%) of treated cells and compared to untreated control cells (Table 2). Range of growth (%) showed the lowest and the highest growth that was found among different cancer cell lines.

The synthesized 5-arylidene-3-(benzothiazol-2-ylamino)-2-thioxo-4-thiazolidones (**2**, **4**, **6-8**) and 5-arylidene-2-(6-methylbenzothiazol-2-ylimino)-4-thiazolidinones (**12**–**16**) displayed moderate (**7**, **12**, **14**) or low (**2**, **4**, **8**, **13**, **15**) activity in the *in vitro* screen on tested cell lines. Of note, that there was observed selective influence of some compounds on several cancer cell lines (Table 2). The compound **4** was highly active on renal cancer RXF 393 cell line (GP = −0.71%), compounds **12** and **14** were highly active on the CNS cancer SF-295 cell line (GP = 22.52% and 4.01%, respectively) and **7** – on the leukemia RPMI-8226 cell line (GP = 20.02%).

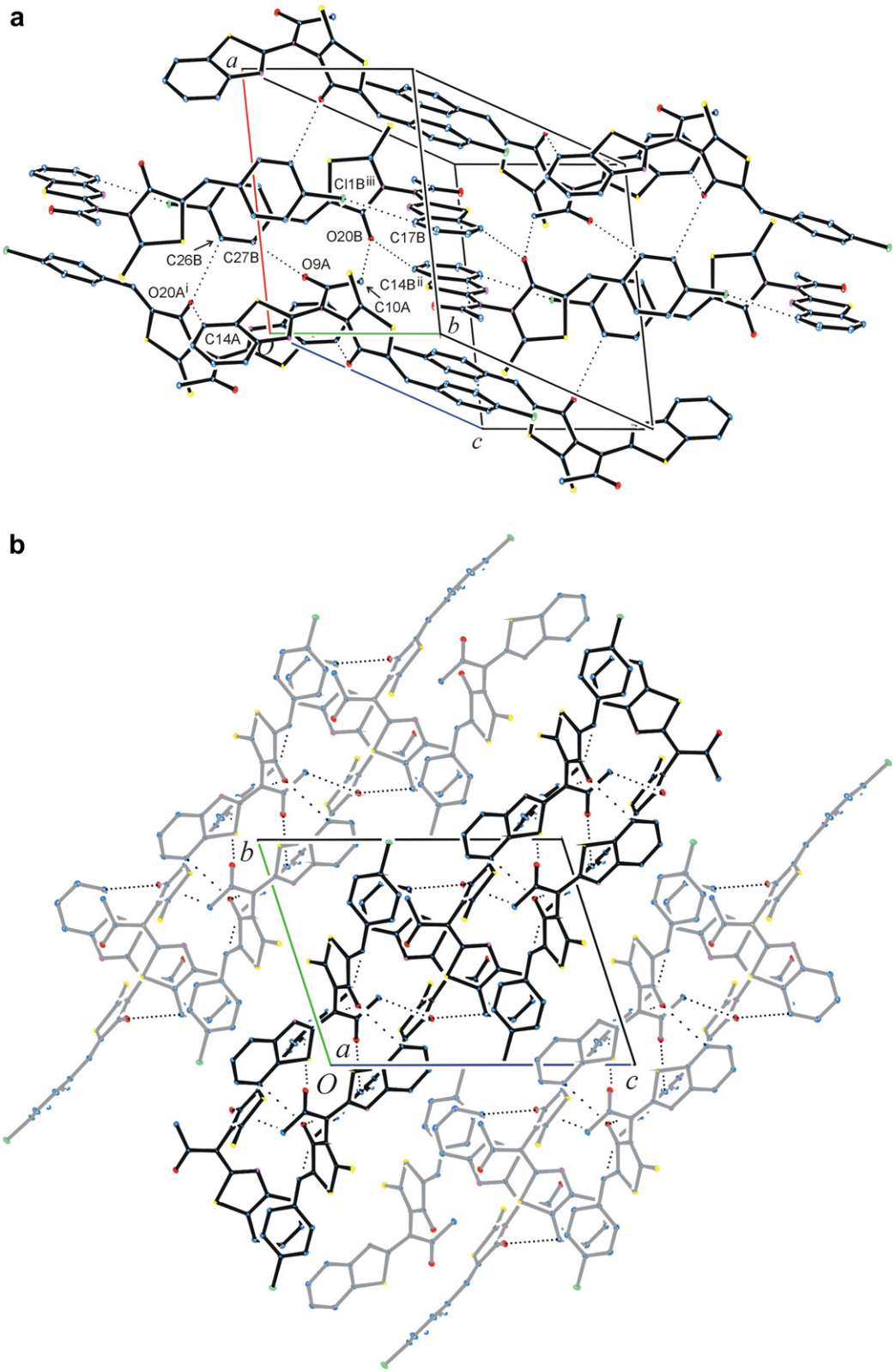


Fig. 3. Part of the molecular packing of **9**, showing (a) the C–H···O and C–H···Cl weak hydrogen bonds and (b) the hydrogen bonded sheets parallel to the (0–11) plane. The symmetry codes are explained in Table 1.

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

$D-H\cdots A$	$D-H$	$H\cdots A$	$D\cdots A$	$D-H\cdots A$
C10A–H10A···O20B	0.96	2.40	3.356(2)	174
C14A–H14A···O20A^{i}	0.93	2.50	3.178(2)	130
C14B^{ii}–H14B^{ii}···O20B	0.93	2.47	3.096(2)	125
C17B–H17B···Cl1B^{iii}	0.93	2.69	3.429(2)	137
C26B–H26B···O20A^{i}	0.93	2.40	3.286(2)	160

Symmetry codes: (i) -x,-y,-z; (ii) 1-x,1-y,1-z; (iii) 1-x,-y,-z.

Finally, compounds **6** and **16** possessed considerable activity against all tested human tumor cell lines and were selected in advanced assay against a panel of approximately sixty tumor cell lines at 10-fold dilutions of five concentrations (100 μM, 10 μM, 1 μM, 0.1 μM and 0.01 μM) [27–31]. The percentage of growth was evaluated spectrophotometrically versus controls not treated with test agents. The 48-h continuous drug exposure protocol followed and SRB (sulforodamine B) protein assay was used to estimate cell viability or growth. Based on the cytotoxicity assays, three anti-tumor activity dose-response parameters were calculated for experimental agents against each cell line: GI_{50} – molar concentration of the compound that inhibits 50% net cell growth; TGI – molar concentration of the compound leading to total inhibition; and LC_{50} - molar concentration of the compound leading to 50% net cell death. Furthermore, mean graph midpoints (MG_MID) were calculated for each of the parameters, giving an average activity parameter over all cell lines for tested compounds. For the calculation of the MG_MID the insensitive cell lines were included with the highest tested concentration (Table 3).

The tested compounds showed a broad spectrum of growth inhibition activity against all human tumor cells with average $lgGI_{50}$/lgTGI values −5.38/−4.45 (**6**) and −4.97/−4.20 (**16**), respectively (Table 3). Selectivity pattern analysis of cell lines by disease origin can definitely affirm selective action of compounds **6** on leukemia cell lines and **16** – on CNS cancer (Fig. 4). These compounds appeared to be the most active against selected individual cell lines with the $logGI_{50}$ varying from −6.34 to −5.00 (Table 3). The compound **6** was found to be a highly active growth inhibitor of the leukemia cell line HL-60 (TB), non-small cell lung cancer cell line NCI-H226, colon cancer line KM-12, CNS cancer cell line SNB-75, ovarian cancer cell line OVCAR-3 and prostate cancer cell line DU-145. The compound **16** showed selectivity on non-small cell lung cancer cell line HOP-92 and CNS cancer cell line SF-539.

NCI web-resources allow to compare selectivity patterns of tested compounds with standard anticancer agents. Successful application of COMPARE algorithm could provide the preliminary information regarding growth inhibition and cell killing mechanism. COMPARE [27,31] analysis was performed for the active compound **6** at GI_{50} level, however obtained correlation coefficients (r) didn't allow to distinguish a cytotoxicity mechanism of tested compounds with high probability. Nevertheless compound **6** showed moderate correlations with rifamycin SV (NSC:S133100, DNA-polymerase inhibitor, $r = 0.531$) [32] and thallicarpine (NSC:S68075 p-glycoprotein inhibitor, $r = 0.516$) [33].

The SAR study revealed that: (1) anticancer activity of compounds **2**, **4**, **6**–**8**, **12**–**16** is sensitive to the nature of substituent in position 5 of 4-thiazolidinone cycle, (2) introduction of 4-chlorophenoxy-N-(4-methoxyphenyl)-acetamide group (compounds **6**, **16**) in 5-position of 4-thiazolidinone core enhanced potency, (3) coupling of 4-thiazolidinone and benzothiazole moieties in a single molecule is a promising approach for exploration anicancer agents, and (4) linking position of benzothiazole fragment (2 or 3) with 4-thiazolidinone core did not essentially influence antitumor activity.

Table 3
The influence of compounds **6** and **16** on the growth of individual tumor cell lines ($logGI_{50} \leq -5.00$).

Compound	Disease	Cell line	$logGI_{50}$	logTGI
6	MG_MID		−5.38	−4.45
	Leukemia	HL-60 (TB)	−5.64	−5.16
	Leukemia	RPMI-8226	−5.27	−4.89
	NSC lung cancer	NCI-H226	−5.53	−5.16
	Colon cancer	KM-12	−5.65	−5.31
	Colon cancer	SW-620	−5.61	−5.20
	CNS cancer	SNB-75	−5.80	−5.02
	CNS cancer	U251	−5.53	−5.03
	Melanoma	LOX IMVI	−5.78	−5.49
	Melanoma	SK-MEL-28	−5.66	−5.35
	Ovarian cancer	OVCAR-3	−5.70	−5.43
	Renal cancer	A498	−5.59	−5.24
	Prostate Cancer	DU-145	−5.68	−5.40
	Breast cancer	BT-549S	−5.52	−5.05
16	MG_MID		−4.97	−4.20
	NSC lung cancer	HOP-92	−6.34	−4.99
	NSC lung cancer	NCI-H23	−5.11	−4.60
	Colon cancer	HCT-116	−5.34	−4.46
	CNS cancer	SF-295	−5.47	−4.81
	CNS cancer	SF-539	−5.75	−5.44
	CNS cancer	SNB-19	−5.26	−4.60
	CNS cancer	U251	−5.30	−4.70
	Melanoma	UACC-62	−5.27	−4.40
	Ovarian cancer	OVCAR-4	−5.22	−4.00
	Ovarian cancer	NC/ADR-RES	−5.17	−4.00
	Renal cancer	ACHN	−5.19	−4.18
	Prostate cancer	PC-3	−5.31	−4.00
	Breast cancer	MCF-7	−5.37	−4.00
	Breast cancer	BT-549	−5.22	−4.56
	Breast cancer	MDA-MB-435	−5.00	−4.46
	Breast cancer	T-47D	−5.32	−4.00

Table 2
Anticancer screening data in concentration 10^{-5} M.

Comp	60 cell lines assay in 1 dose 10^{-5} M conc				Active (selected for 5-dose 60 cell lines assay)
	Mean growth %	Range of growth %	The most sensitive cell line	growth % of the most sensitive cell line	
2	98.52	77.27 to 121.76	UO-31 (Renal Cancer)	77.27	Inactive
4	97.65	−0.71 to 129.24	RXF 393 (Renal Cancer)	−0.71	Inactive
6	50.36	−71.48 to 117.53	SR (Leukemia)	−71.48	Active
7	74.74	20.02 to 115.14	RPMI-8226 (Leukemia)	20.02	Inactive
8	107.49	78.63 to 131.69	UO-31 (Renal Cancer)	78.63	Inactive
12	69.02	22.52 to 111.91	SF–295 (CNS Cancer)	22.52	Inactive
13	105.63	80.36 to 148.60	UO-31 (Renal Cancer)	80.36	Inactive
14	56.54	4.01 to 116.20	SF–295 (CNS Cancer)	4.01	Inactive
15	91.85	51.40 to 141.04	HOP-92 (Non-Small Cell Lung Cancer)	51.40	Inactive
16	−36.88	−86.51 to 77.08	SNB-75 (CNS Cancer)	−86.51	Active

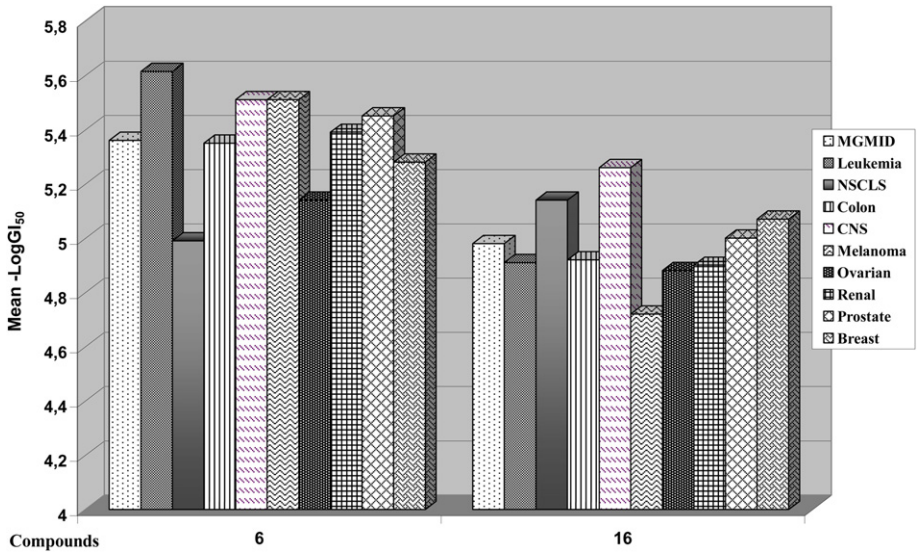


Fig. 4. Anticancer selectivity pattern of the most active compounds 6 and 16.

3. Conclusions

In the present paper, sixteen thiazolidinones with benzothiazole fragment were described. Ten of synthesized compounds were tested and five of them displayed moderate or considerable antitumor activity against leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers cell lines.

In conclusion, these preliminary results allowed to identify the most active compounds **6** and **16**. Especially 2-{2-[3-(benzothiazol-2-ylamino)-4-oxo-2-thioxothiazolidin-5-ylidenemethyl]-4-chlorophenoxy}-N-(4-methoxyphenyl)-acetamide (**6**) could be a prospective antitumor agent (average $logGI_{50}$ and logTGI values −5.38 and −4.45 respectively). The obtained results prove the necessity of further investigations in order to clarify the features underlying the antitumor potential of tested compounds.

4. Experimental

4.1. Materials and methods

The starting (benzothiazole-2-yl)hydrazine [34], trithiocarbonyl diglycolic acid [20], 6-methyl-2-aminobenzothiazole [35], carbethoxymethylthio-2-thiazoline-4-one [3,21] were obtained according to methods described previously.

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 a Perkin–Elmer 2400 CHN analyzer. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values. The ^{1}H NMR spectra were recorded on Varian Gemini 300 MHz and ^{13}C NMR spectra on Varian Mercury-400 100 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.

4.2. Chemistry

4.2.1. Synthesis of 3-(benzothiazol-2-ylamino)-2-thioxo-4-thiazolidinone (*1*)

A mixture of 50 mmol (benzothiazole-2-yl)hydrazine and 50 mmol trithiocarbonyl diglycolic acid was refluxed in 30 ml of ethanol during 5 h. After cooling the reaction mixture was poured into cold water. A precipitated solid was filtered, dried and recrystallized in turn with AcOH: H_2O mixture (1:1) and chloroform.

Yield 78%, mp 137–140 °C. IR [cm^{-1}]: 3512, 3448, 3112, 2968, 2896, 1648 (CO), 1588, 1496 (C=N), 1456, 1424, 1320, 1248, 1032 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ [ppm] 12.40, 11.60 (2*br.s, s, 1H, NH); 7.70 (m, 1H, arom.); 7.34 (m, 2H, arom.); 7.16 (m, 1H, arom.); 4.39 (s, 2H, CH_2). Calcd. for $C_{10}H_7N_3OS_3$: C, 42.69; H, 2.51; N, 14.93; Found: C, 42.50; H, 2.60; N, 15.00%.

4.2.2. General procedure for synthesis of 5-arylidene-3-(benzothiazol-2-ylamino)–2-thioxo-4-thiazolidinones (*2*–*8*) and N-(benzothiazol-2-yl)-N-(5-arylidene-4-oxo-2-thioxothiazolidin-3-yl)-acetamides (*9*, *10*)

A mixtures of compound **1** (3 mmol), appropriate aldehyde (3.3 mmol) and anhydrous sodium acetate (3 mmol) were refluxed for 2 h in glacial acetic acid (10 ml) (**2**–**8**) or in a mixture of 10 ml glacial acetic acid with 5 ml acetic anhydride (**9**, **10**). Obtained powders were filtered off, washed with methanol and recrystallized with DMF: ethanol (1:2) mixture.

4.2.2.1. 5-(4-Diethylaminobenzylidene)-3-(benzothiazol-2-ylamino)-2-thioxo-4-thiazolidinone (2). Yield 63%, mp 236–237 °C. IR [cm^{-1}]: 2976, 2864, 1704 (CO), 1668, 1608, 1568, 1552 (C=C), 1512 (C=N), 1348, 1256, 1224, 1188, 1152, 1096 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ [ppm] 12.53, 11.77 (2*br.s, s, 1H, NH); 7.77 (br.s, 1H, = CH); 7.68 (m, 1H, arom.); 7.53 (d, 2H, J = 8.6 Hz, arom.); 7.32 (m, 2H, arom.); 7.15 (m, 1H, arom.); 6.84 (d, 2H, J = 8.6 Hz, arom.); 3.47 (q, 4H, J = 6.8 Hz, 2*CH_2); 1.46 (t, 6H, J = 6.8 Hz, 2*CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 190.4 (C=S), 164.3 (C=O), 150.6, 138.2, 136.5, 134.8, 127.4, 123.4, 119.9, 112.7, 44.7 (CH_2), 13.1 (CH_3). Calcd. for $C_{21}H_{20}N_4OS_3$: C, 57.25; H, 4.58; N, 12.72; Found: C, 57.60; H, 4.70; N, 12.90%.

4.2.2.2. 5-(4-Dimethylaminobenzylidene)-3-(benzothiazol-2-ylamino)-2-thioxothiazolidinone (3). Yield 59%, mp 249–250 °C. IR [cm^{-1}]: 2984, 2936, 2864, 1744 (CO), 1668, 1608, 1564 (C=C), 1516 (C=N), 1440, 1376, 1348, 1256, 1224, 1188, 1092 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ [ppm] 12.48, 11.60 (2*br.s, s, 1H, NH); 7.78 (br.s, 1H, = CH); 7.70 (m, 1H, arom.); 7.53 (d, 2H, J = 8.0 Hz, arom.); 7.34

(m, 2H, arom.); 7.16 (m, 1H, arom.); 6.88 (d, 2H, J = 8.0 Hz, arom.); 3.07 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 190.1 (C=S), 164.2 (C=O), 153.4, 149.9, 144.2, 137.9, 123.3, 113.1, 39.3 (CH_3). Calcd. for $C_{19}H_{16}N_4OS_3$: C, 55.32; H, 3.91; N, 13.58; Found: C, 55.70; H, 4.10; N, 13.80%.

*4.2.2.3. 5-(3-Methoxy-4-hydroxybenzylidene)-3-(benzothiazol-2-ylamino)-2-thioxo-4-thiazolidinone (**4**).* Yield 78%, mp 208–210 °C. IR [cm^{-1}]: 3136 (OH), 3096 (NH), 2920, 2904, 1712 (CO), 1680, 1624, 1608, 1580 (C=C), 1532, 1504 (C=N), 1472, 1336, 1256, 1216, 1128 (C=S).^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ [ppm] 11.85, 10.08 (2*br.s, 1H, NH); 8.92 (br.s, 1H, OH); 7.82 (br.s, 1H, = CH); 7.68 (d, J = 7.6 Hz, 1H, arom.); 7.57 (m, 1H, arom.); 7.51 (d, J = 6.8 Hz, 1H, arom.); 7.30–7.32 (m, 1H, arom.), 7.25 (s, 1H, arom); 7.14–7.20 (m, 2H, arom); 3.07 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 190.4 (C=S), 162.1 (C=O), 152.5, 151.3, 148.9, 131.8, 130.9, 127.7, 125.6, 115.5, 56.3 (CH_3). Calcd. for $C_{18}H_{13}N_3O_3S_3$: C, 52.03; H, 3.15; N, 10.11; Found: C, 52.20; H, 3.10; N, 10.45%.

*4.2.2.4. 5-(4-Chlorobenzylidene)-3-(benzothiazol-2-ylamino)-2-thioxo-4-thiazolidinone (**5**).* Yield 68%, mp 264–266 °C. IR [cm^{-1}]: 2848, 2296, 1808, 1768, 1716 (CO), 1696, 1588 (C=C), 1524 (C=N), 1496, 1472, 1240, 1208, 1088 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ [ppm] 12.50, 11.60, 11.00 (3*br.s, 1H, NH); 7.94 (br.s, 1H, = CH); 7.73 (d, J = 6.8 Hz, 2H, arom.); 7.70 (m, 1H, arom.); 7.62 (d, J = 6.8 Hz, 2H, arom.); 7.32 (m, 2H, arom.), 7.15 (m, 1H, arom.). ^{13}C NMR (100 MHz, DMSO-d_6): δ 190.3 (C=S), 164.1 (C=O), 158.1, 149.2, 136.7, 133.3, 132.5, 130.4, 127.5, 123.6. Calcd. for $C_{17}H_{10}ClN_3OS_3$: C, 50.55; H, 2.50; N, 10.40; Found: C, 50.20; H, 2.15; N, 10.60%.

*4.2.2.5. 2-{2-[3-(Benzothiazol-2-ylamino)-4-oxo-2-thioxothiazolidin-5-ylidenemethyl]-4-chlorophenoxy}-N-(4-methoxyphenyl)-acetamide (**6**).* Yield 73%, mp 151–152 °C. IR [cm^{-1}]: 1708 (CO), 1688, 1680 (CO), 1584 (C=C), 1568, 1512 (C=N), 1474, 1228, 1040 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ [ppm] 11.84, 10.12, 10.00 (br.s, 3*s, 2H, 2*NH); 8.05 (s, 1H, = CH); 7.46–7.58 (m, 5H, arom.); 7.30 (br.s, 2H, arom.); 7.12 (m, 2H, arom.); 6.86 (d, 2H, J = 9.1 Hz, arom.); 4.92 (s, 2H, CH_2); 3.68 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 190.7 (C=S), 165.8 (C=O), 156.4 (C=O), 156.2, 133.1, 132.0, 126.1, 121.8, 115.6, 114.6, 68.1 (CH_2), 55.8 (CH_3). Calcd. for $C_{17}H_{10}ClN_3OS_3$: C, 50.55; H, 2.50; N, 10.40; Found: C, 50.20; H, 2.15; N, 10.60%. Calcd. for $C_{26}H_{19}ClN_4O_4S_3$: C, 53.56; H, 3.28; N, 9.61; Found: C, 53.20; H, 3.15; N, 9.90%.

*4.2.2.6. 4-(2-{2-[3-(Benzothiazol-2-ylamino)-4-oxo-2-thioxothiazolidin-5-ylidenemethyl]-4-chlorophenoxy}-acetylamino)-benzoic acid ethyl ester (**7**).* Yield 71%, mp 180–182 °C. IR [cm^{-1}]: 1704 (CO), 1600 (C=C), 1528 (C=N), 1472, 1408, 1368, 1244, 1044 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ [ppm] 11.85, 10.58 (br.s, s, 2H, 2*NH); 8.07 (br.s, 1H, = CH), 7.91 (d, 2H, J = 8.7 Hz, arom.), 7.74 (d, 2H, J = 8.7 Hz, arom.), 7.70–7.48 (m, 3H, arom.), 7.33 (t, 1H, J = 8.9 Hz, arom.), 7.18–7.13 (m, 3H, arom.); 5.01 (s, 2H, CH_2); 4.24 (q, 2H, J = 7.0 Hz, CH_2); 1.24 (t, 3H, J = 7.1 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 191.5 (C=S), 167.0 (C=O), 165.9 (C=O), 156.4, 143.3, 130.9, 126.2, 125.3, 119.5, 61.2 (CH_2), 14.9 (CH_3). Calcd. for $C_{28}H_{21}ClN_4O_5S_3$: C, 53.80; H, 3.39; N, 8.96; Found: C, 54.00; H, 3.25; N, 9.10%.

*4.2.2.7. 2-{4-[3-(Benzothiazol-2-ylamino)-4-oxo-2-thioxothiazolidin-5-ylidenemethyl]-2-methoxyphenoxy}-N-(4-methoxyphenyl)-acetamide (**8**).* Yield 68%, mp 232–234 °C. IR [cm^{-1}]: 1708 (CO), 1696, 1596 (C=C), 1524 (C=N), 1472, 1408, 1236, 1108 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ 11.84, 10.02 (br.s, s, 2H, 2*NH); 7.88 (br.s, 1H, = CH); 7.69 (m, 1H, arom.); 7.53 (d, 2H, J = 9.0 Hz, arom.); 7.33–7.26 (m, 4H, arom.); 7.16–7.09 (m, 2H, arom.); 6.90 (d, J = 9.0 Hz, 2H, arom.); 4.79 (s, 2H, CH_2); 3.89 (s, 3H, OCH_3); 3.70 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.6 (C=S), 166.3 (C=O), 156.3, 151.1, 150.1, 132.2, 127.1, 125.7, 123.3, 121.7, 115.4, 115.2, 68.3 (CH_2), 56.4 (CH_3), 55.8 (CH_3). Calcd. for $C_{27}H_{22}N_4O_5S_3$: C, 56.04; H, 3.83; N, 9.68; Found: C, 55.75; H, 3.75; N, 9.40%.

*4.2.2.8. N-(Benzothiazol-2-yl)-N-[5-(4-chlorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]-acetamide (**9**).* Yield 71%, mp 209–211 °C. IR [cm^{-1}]: 1744 (CO), 1680, 1632, 1600 (C=C), 1584, 1504 (C=N), 1440, 1368, 1296, 1264, 1208, 1128, 1092 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ [ppm] 8.13 (s, 1H, = CH), 8.06 (d, 1H, J = 7.9 Hz, arom.), 7.81 (d, 2H, J = 8.2 Hz, arom.), 7.73 (d, 1H, J = 8.0 Hz, arom.), 7.67 (d, 2H, J = 8.2 Hz, arom.), 7.46 (t, 1H, J = 7.3 Hz, arom.), 7.38 (t, 1H, J = 7.6 Hz, arom.), 2.37 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.6 (C=S), 171.3 (C=O), 163.2 (C=O), 155.1, 147.5, 137.3, 136.2, 133.5, 132.1, 130.5, 127.4, 125.5, 122.8, 122.3, 119.9, 21.6 (CH_3). Calcd. for $C_{19}H_{12}ClN_3O_2S_3$: C, 51.17; H, 2.71; N, 9.42; Found: C, 51.35; H, 2.75; N, 9.20%.

*4.2.2.9. N-(Benzothiazol-2-yl)-N-[5-(3-methoxy-4-acetoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl]-acetamide (**10**).* Yield 74%, mp 176–178 °C. IR [cm^{-1}]: 2928, 2856, 1744 (CO), 1728 (CO), 1672, 1600 (C=C), 1552, 1504 (C=N), 1368, 1352, 1308, 1264, 1248, 1232, 1192, 1168, 1124 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ [ppm] 8.14 (s, 1H, = CH), 8.07 (d, 1H, J = 6.5 Hz, arom.), 7.73 (d, 1H, J = 7.2 Hz, arom.), 7.54 (s, 1H, arom.), 7.38–7.34 (m, 4H, arom.), 3.87 (s, 3H, OCH_3), 2.36 (s, 3H, CH_3), 2.29 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 189.9 (C=S), 171.4, 168.9 (C=O), 165.9 (C=O), 163.3 (C=O), 155.1 (C=N), 152.2, 147.5, 142.5, 137.1, 133.5, 132.1, 126.2, 125.5, 124.1, 122.8, 122.3, 119.4, 115.4, 56.8 (CH_3), 25.5 (CH_3), 21.6 (CH_3). Calcd. for $C_{22}H_{17}N_3O_5S_3$: C, 52.89; H, 3.43; N, 8.41; Found: C, 52.55; H, 3.50; N, 8.20%.

*4.2.3. Synthesis of 2-(6-methylbenzothiazol-2-ylimino)-4-thiazolidinone (**11**)*

Method A. A mixture of 2-carbethoxymethylthio-2-thiazoline-4-one (20 mmol) and 6-methyl-2-aminobenzothiazole (20 mmol) was refluxed for 1 h in 150 ml of ethanol. After cooling to the room temperature precipitated light brown powder was filtered off, washed with methanol and recrystallized with glacial acetic acid.

Method B. A mixture of 2-chloro-N-(6-methylbenzothiazol-2-yl)-acetamide (20 mmol) and ammonium thiocyanate (40 mmol) was refluxed for 3 h in acetone (20 ml) during 5 h. After the reaction was completed, the mixture was cooled. The precipitate was collected by filtration, washed with water and recrystallized with glacial acetic acid.

*4.2.3.1. 2-(6-Methylbenzothiazol-2-ylimino)-4-thiazolidinone (**11**).* Yields 74% (method A), 59% (method B), mp 243–245 °C. IR [cm^{-1}]: 3050 (NH), 1720 (CO), 1584 (C=N), 1552, 1448, 1216, 1160. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ [ppm] 12.25 (br.s, 1H, NH), 7.74 (s, 1H, arom.), 7.64 (d, 1H, J = 8.0 Hz, arom.), 7.25 (d, 1H, J = 8.4 Hz, arom.), 4.02 (s, 2H, CH_2), 2.39 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 174.9 (C=O), 166.3 (C=N), 149.5 (C=N), 134.5, 133.8, 128.3, 122.2, 121.7, 35.8 (CH_2), 21.7 (CH_3). Calcd. for $C_{11}H_9N_3OS_2$: C, 50.17; H, 3.44; N, 15.96; Found: C, 50.05; H, 3.50; N, 16.00%.

*4.2.4. General procedure for synthesis of 5-arylidene-2-(6-methylbenzothiazol-2-ylimino)-4-thiazolidinones (**12**–**16**)*

A mixture of compound **11** (3 mmol), appropriate aldehyde (4 mmol) and anhydrous sodium acetate (3 mmol) was refluxed for 3 h in glacial acetic acid (30 ml). Powder obtained after cooling was filtered off, washed with methanol and recrystallized with DMF: ethanol (1:2).

*4.2.4.1. 5-(4-Diethylaminobenzylidene)-2-(6-methylbenzothiazol-2-ylimino)-4-thiazolidinone (**12**).* Yields 68%, mp 205–207 °C. IR [cm^{-1}]: 2976 (NH), 2912, 1704 (CO), 1680, 1632, 1600 (C=C), 1580, 1504, 1472, 1344, 1300, 1268, 1192, 1144, 1080. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ [ppm] 12.62 (br.s, 1H, NH), 7.83 (d, 1H, J = 8.1 Hz, arom.), 7.76 (s, 1H, arom.), 7.62 (s, 1H, = CH), 7.53 (d, 2H, J = 8.7 Hz, arom.), 7.29 (d, 1H, J = 8.3 Hz, arom.), 6.63 (d, 2H, J = 8.7 Hz, arom.), 3.45 (q, 4H, J = 6.9 Hz, 2*CH_2), 2.42 (s, 3H, CH_3), 1.12 (t, 6H, J = 6.9 Hz, 2*CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.3 (C=O), 159.5, 149.9, 134.8, 133.8, 128.4, 122.5, 122.0, 120.1, 116.3, 112.3, 44.5 (CH_2), 21.6 (CH_3), 12.9 (CH_3). Calcd. for $C_{22}H_{22}N_4OS_2$: C, 62.53; H, 5.25; N, 13.26; Found: C, 62.25; H, 5.50; N, 13.05%.

*4.2.4.2. 5-(4-Methoxybenzylidene)-2-(6-methylbenzothiazol-2-ylamino)-4-thiazolidinone (**13**).* Yields 82%, mp 185–187 °C. IR [cm^{-1}]: 3144, 3008 (NH), 2944, 1700 (CO), 1592 (C=C), 1512 (C=N), 1452, 1348, 1260, 1176, 1152, 1024. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ [ppm] 12.77 (br.s, 1H, NH), 7.80 (d, 1H, J = 8.0 Hz, arom.), 7.77 (s, 1H, arom.), 7.68 (s, 1H, = CH), 7.66 (d, 2H, J = 8.6 Hz, arom.), 7.31 (d, 1H, J = 7.7 Hz, arom.), 7.16 (d, 2H, J = 8.6 Hz, arom.), 3.83 (s, 3H, OCH_3), 2.41 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.9 (C=O), 161.7, 158.9, 149.5, 134.7, 133.9, 133.3, 128.5, 126.4, 122.3, 122.1, 121.7, 115.6, 59.2 (CH_3), 21.7 (CH_3). Calcd. for $C_{19}H_{15}N_3O_2S_2$: C, 59.82; H, 3.96; N, 11.02; Found: C, 60.00; H, 4.10; N, 11.05%.

*4.2.4.3. 5-(4-Chlorobenzylidene)-2-(6-methylbenzothiazol-2-ylimino)-4-thiazolidinone (**14**).* Yields 78%, mp 269–270 °C. IR [cm^{-1}]: 3104 (NH), 1712 (CO), 1588 (C=C), 1560 (C=N), 1488, 1456, 1328, 1152, 1088. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ [ppm] 12.89 (br.s, 1H, NH), 7.72–7.78 (m, 2H, arom.), 7.72 (s, 1H, = CH), 7.66 (d, 2H, J = 8.4 Hz, = CH), 7.59 (d, 2H, J = 8.4 Hz, arom.), 7.29 (d, 1H, J = 8.2 Hz, arom.), 2.42 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.7 (C=O), 158.4, 149.5, 135.6, 134.8, 134.0, 132.9, 132.5, 131.8, 130.2, 128.5, 125.8, 122.3, 21.7 (CH_3). Calcd. for $C_{18}H_{12}ClN_3OS_2$: C, 56.03; H, 3.13; N, 10.89; Found: C, 55.90; H, 3.00; N, 11.00%.

*4.2.4.4. 2-{4-Chloro-2-[2-(6-methylbenzothiazol-2-ylimino)-4-oxothiazolidin-5-ylidenemethyl]-phenoxy}-acetamide (**15**).* Yields 69%, mp 211–213 °C. IR [cm^{-1}]: 3392, 3216 (NH), 2864, 2480, 1712 (CO), 1656, 1592 (C=N), 1564, 1484, 1336, 1232, 1156. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ [ppm] 12.91 (br.s, 1H, NH), 7.72 (d, 1H, J = 8.4 Hz, arom.), 7.61 (s, 1H, = CH), 7.56 (s, 1H, arom.), 7.55 (s, 2H, NH_2), 7.53 (d, 1H, J = 8.5 Hz, arom.), 7.42 (s, 1H, arom.), 7.30 (d, J = 8.4 Hz, 1H), 7.04 (d, J = 8.5 Hz, 1H), 4.66 (s, 2H, CH_2), 2.42 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.9 (C=O), 168.0 (C=O), 158.7, 156.4, 149.5, 134.9, 134.1, 132.1, 128.8, 127.2, 126.9, 125.8, 124.9, 122.4, 122.0, 115.3, 67.8 (CH_2), 21.6 (CH_3). Calcd. for $C_{20}H_{15}ClN_4O_3S_2$: C, 52.34; H, 3.29; N, 12.21; Found: C, 52.10; H, 3.50; N, 12.05%.

*4.2.4.5. 2-{4-Chloro-2-[2-(6-methylbenzothiazol-2-ylimino)-4-oxothiazolidin-5-ylidenemethyl]-phenoxy}-N-(4-methoxyphenyl)-acetamide (**16**).* Yields 75%, mp 278–279 °C. IR [cm^{-1}]: 2840, 1712 (CO), 1680, 1588 (C=C), 1512 (C=N), 1484, 1336, 1248, 1152. ^{1}H NMR (300 MHz, DMSO-d_6+CCl_4): δ [ppm] 12.92 (br.s, 1H, NH), 10.09 (s, 1H, NH), 7.71 (d, 1H, J = 8.2 Hz, arom.), 7.62 (s, 1H, = CH), 7.53–7.58 (m, 2H, arom.), 7.54 (s, 1H, arom.), 7.52 (d, 2H, J = 9.0 Hz, arom.), 7.27 (d, 1H, J = 8.4 Hz, arom.), 7.14 (d, 1H, J = 8.8 Hz, arom.), 6.88 (d, 2H, J = 9.0 Hz, arom.), 4.90 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3), 2.41 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.8 (C=O), 165.9 (C=O), 156.4, 156.2, 149.4, 134.8, 134.0, 132.1, 128.8, 128.6, 126.9, 125.8, 124.8, 122.4, 121.9, 121.7, 115.5, 114.6, 68.3 (CH_2), 55.8 (C=O), 21.8 (CH_3). Calcd. for $C_{27}H_{21}ClN_4O_4S_2$: C, 57.39; H, 3.75; N, 9.91; Found: C, 57.10; H, 4.00; N, 10.05%.

4.3. Pharmacology

A primary anticancer assay was performed at approximately a sixty human tumor cell lines panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda [27–31]. Tested compounds were added to the culture at a single concentration (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. The 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, (Tz), control growth in the absence of drug, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

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

Three dose-response parameters were calculated for each compound. Growth inhibition of 50% (GI_{50}) was calculated from $[(Ti-Tz)/(C-Tz)] \times 100 = 50$, 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 $Ti = Tz$. 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 $[(Ti-Tz)/Tz] \times 100 = -50$. 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 more 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 $\leq$4 were declared to be active.

4.4. Crystal structure determination of **9**

Crystal data: $C_{19}H_{12}ClN_3O_2S_3$, M_r = 445.95, triclinic, space group P-1, a = 11.3492(4), b = 11.8104(5), c = 15.8699(7) Å, α = 105.378(4), β = 107.260(4), γ = 94.802(3)°, V = 1927.95(13) Å^3, T = 110(1) K, Z = 4.

Data collection: A dark-yellow block crystal of 0.55 × 0.30 × 0.24 mm was used to record 20 890 (Mo$K\alpha$ radiation, θ_{max} = 29.0°) intensities on a Xcalibur A diffractometer. The crystal was positioned at 55 mm from the CCD camera. 392 Frames were measured at 1° intervals with a counting time of 20 s. The data were corrected for Lorentz and polarization effects. Multi-scan absorption correction has been applied too. The minimum and maximum transmissions were 0.9183 and 1.0000. Data reduction and analysis were carried out with the Oxford Diffraction programs [36]. The 8977 total unique reflections (R(int) = 0.013) were used for further calculations. All H atoms were placed in geometrically calculated

positions and refined using a riding model, with C–H = 0.93 ($C_{sp}2H$) and 0.96 Å (CH_3) and $U_{iso}(H) = 1.2U_{eq}(C)$ or $1.5U_{eq}(C)$ for methyl H atoms. The methyl groups were refined as a rigid groups, which were allowed to rotate.

Structure solution and refinement: The structure was solved by the direct methods using the program SHELXS-97 [37] and refinement was done against F^2 for all data using SHELXL-97 [37]. The final refinement converged with $R = 0.0322$ (for 7754 data with $F^2 > 4\sigma(F^2)$), $wR = 0.0932$ (on F^2 for all data), and $S = 1.064$ (on F^2 for all data). The largest difference peak and hole were 0.982 and −0.829 eÅ^{-3}. The molecular illustration was drawn using ORTEP-3 for Windows [38]. Software used to prepare material for publication was WINGX [39] and PLATON [40].

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 752 285).

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References

[1] R. Lesyk, B. Zimenkovsky, 4-Thiazolidones: centenarian history, current status and perspectives for modern organic and medicinal chemistry, Curr. Org. Chem. 8 (2004) 1547–1577.
[2] R. Lesyk, O. Vladzimirska, S. Holota, L. Zaprutko, A. Gzella, New 5-substituted thiazolo[2,3-d][1,2,4]triazol-6-ones. Synthesis and anticancer evaluation, Eur. J. Med. Chem. 42 (2007) 641–648.
[3] D. Havrylyuk, B. Zimenkovsky, O. Vasylenko, L. Zaprutko, A. Gzella, R. Lesyk, Synthesis of novel thiazolone-based compounds containing pyrazoline moiety and evaluation of their anticancer activity, Eur. J. Med. Chem. 44 (2009) 1396–1404.
[4] D. Havrylyuk, B. Zimenkovsky, R. Lesyk, Synthesis and anticancer activity of novel nonfused bicyclic thiazolidinone derivatives, Phosphorus, Sulfur, and Silicon 184 (2009) 638–650.
[5] D. Kaminskyy, B. Zimenkovsky, R. Lesyk, Synthesis and in vitro anticancer activity of 2,4-azolidinedione-acetic acids derivatives, Eur. J. Med. Chem. 44 (2009) 3627–3636.
[6] J.H. Ahn, S.J. Kim, W.S. Park, S.Y. Cho, J.D. Ha, S.S. Kim, S.K. Kang, D.G. Jeong, S.-K. Jung, S.-H. Lee, H.M. Kim, S.K. Park, K.H. Lee, C.W. Lee, S.E. Ryu, J.-K. Choi, Synthesis and biological evaluation of rhodanine derivatives as PRL-3 inhibitors, Bioorg. Med. Chem. Lett. 16 (2006) 2996–2999.
[7] H. Park, S.-K. Jung, D.G. Jeong, S.E. Ryu, S.J. Kim, Discovery of novel PRL-3 inhibitors based on the structure-based virtual screening, Bioorg. Med. Chem. Lett. 18 (2008) 2250–2255.
[8] A. Geronikaki, P. Eleftheriou, P. Vicini, I. Alam, A. Dixit, A.K. Saxena, 2-Thiazolylimino/heteroarylimino-5-arylidene-4-thiazolidinones as new agents with SHP-2 inhibitory action, J. Med. Chem. 51 (2008) 5221–5228.
[9] N.S. Cutshall, C. O'Day, M. Prezhdo, Rhodanine derivatives as inhibitors of JSP-1, Bioorg. Med. Chem. Lett. 15 (2005) 3374–3379.
[10] P.H. Carter, P.A. Scherle, J.A. Muckelbauer, M.E. Voss, R.-Q. Liu, L.A. Thompson, A.J. Tebben, K.A. Solomon, Y.C. Lo, Z. Li, P. Strzemienski, G. Yang, N. Falahatpisheh, M. Xu, Z. Wu, N.A. Farrow, K. Ramnarayan, J. Wang, D. Rideout, V. Yalamoori, P. Domaille, D.J. Underwood, J.M. Trzaskos, S.M. Friedman, R.C. Newton, C.P. Decicco, Photochemically enhanced binding of small molecules to the tumor necrosis factor receptor-1 inhibits the binding of TNF-α, Proc. Natl. Acad. Sci. U.S.A 98 (21) (2001) 11879–11884.
[11] A. Degterev, A. Lugovskoy, M. Cardone, B. Mulley, G. Wagner, T. Mitchison, J. Yuan, Identification of small-molecule inhibitors of interaction between the BH3 domain and Bcl-xl, Nat. Cell Biol. 3 (2) (2001) 173–182.
[12] R. Dayam, F. Aiello, J. Deng, Y. Wu, A. Garofalo, X. Chen, N. Neamati, Discovery of small molecule integrin $\alpha_v\beta_3$ antagonists as novel anticancer agents, J. Med. Chem. 49 (2006) 4526–4534.
[13] W. Zheng, A. Degterev, E. Hsu, J. Yuan, C. Yuan, Structure–activity relationship study of a novel necroptosis inhibitor, necrostatin-7, Bioorg. Med. Chem. Lett. 18 (2008) 4932–4935.
[14] S.H.L. Kok, R. Gambari, C.H. Chui, M.C.W. Yuen, E. Lin, R.S.M. Wong, F.Y. Lau, G.Y.M. Cheng, W.S. Lam, S.H. Chan, K.H. Lam, C.H. Cheng, P.B.S. Lai, M.W.Y. Yu, F. Cheung, J.C.O. Tang, A.S.C. Chan, Synthesis and anti-cancer activity of benzothiazole containing phthalimide on human carcinoma cell lines, Bioorg. Med. Chem. 16 (2008) 3626–3631.
[15] X. Wang, K. Sarris, K. Kage, D. Zhang, S.P. Brown, T. Kolasa, C. Surowy, O.F. El Kouhen, S.W. Muchmore, J.D. Brioni, A.O. Stewart, Synthesis and evaluation of benzothiazole-based analogues as novel, potent, and selective fatty acid amide hydrolase inhibitors, J. Med. Chem. 52 (2009) 170–180.
[16] E.Y. Song, N. Kaur, M.-Y. Park, Y. Jin, K. Lee, G. Kim, K.Y. Lee, J.S. Yang, J.H. Shin, K.-Y. Nam, K.T. No, G. Han, Synthesis of amide and urea derivatives of benzothiazole as Raf-1 inhibitor, Eur. J. Med. Chem. 43 (2008) 1519–1524.
[17] C.-H. Zheng, Y.-J. Zhou, J. Zhu, H.-T. Ji, J. Chen, Y.-W. Li, C.-Q. Sheng, J.-G. Lu, J.-H. Jiang, H. Tang, Y.-L. Song, Construction of a three-dimensional pharmacophore for Bcl-2 inhibitors by flexible docking and the multiple copy simultaneous search method, Bioorg. Med. Chem. 15 (2007) 6407–6417.
[18] B. Zimenkovsky, G. Kazmirchuk, L. Zaprutko, A. Paraskiewicz, E. Melzer, R. Lesyk, New 5-arylidene-4-thiazolidinones and their anticancer activity, Ann. Polish Chem. Soc. 1 (2005) 69–72.
[19] M. Kawakami, K. Koya, T. Ukai, N. Tatsuta, A. Ikegawa, K. Ogawa, T. Shishido, L.B. Chen, Structure - activity of novel rhodacyanine dyes as antitumor agents, J. Med. Chem. 41 (1998) 130–142.
[20] I. Nektegayev, R. Lesyk, 3-Oxyarylthiazolidones-4 and their choleretic activity, Sci. Pharm. 67 (1999) 227–230.
[21] R. Lesyk, B. Zimenkovsky, I. Subtelna, I. Nektegayev, G. Kazmirchuk, Synthesis and antinflammatory activity of some 2-arylamino-2-thiazoline-4-ones, Acta Poloniae Pharmaceutica. Drug Res. 6 (2003) 457–466.
[22] K. Popov-Pergal, Z. Cekovic, M. Pergal, Condensation of 2,4-dioxotetrahydro-1,3-thiazole with aromatic aldehydes, J. Gen. Chem. USSR 61 (1991) 1958–1962.
[23] M.M. Jotani, B.B. Baldaniya, (2Z)-Ethyl 2-(4-chlorobenzylidene)-7-methyl-3-oxo-5-phenyl-2,3-dihydro-5H-1,3-thiazolo[3,2-a]pyrimidine-6-carboxylate, Acta Cryst E62 (2006) o5871.
[24] F.H. Allen, O. Kennard, D.G. Watson, L. Brammer, A.G. Orpen, R. Taylor, Tables of bond lengths determined by X-ray and neutron diffraction. Part 1. Bond lengths in organic compounds, J. Chem. Soc. Perkin Trans. 2 (1987) 1–19.
[25] P. Vicini, A. Geronikaki, M. Incerti, F. Zani, J. Dearden, M. Hewitt, 2-Heteroarylimino-5-benzylidene-4-thiazolidinones analogues of 2-thiazolylimino-5-benzylidene-4-thiazolidinones with antimicrobial activity: synthesis and structure–activity relationship, Bioorg. Med. Chem. 16 (2008) 3714–3724.
[26] K. Omar, A. Geronikaki, P. Zoumpoulakis, C. Camoutsis, M. Socovic, A. Ciric, J. Glamoclija, Novel 4-thiazolidinone derivatives as potential antifungal and antibacterial drugs, Bioorg. Med. Chem. 18 (2010) 426–432.
[27] http://dtp.nci.nih.gov.
[28] A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo, M. Boyd, Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines, J. Nat. Cancer Inst. 83 (11) (1991) 757–766.
[29] M.R. Boyd, K.D. Paull, Some practical considerations and applications of the national cancer institute in vitro anticancer drug discovery screen, Drug Dev. Res. 34 (1995) 91–109.
[30] M.R. Boyd, in: B.A. Teicher (Ed.), Cancer Drug Discovery and Development, 2, Humana Press, 1997, pp. 23–43.
[31] R.H. Shoemaker, The NCI60 human tumour cell line anticancer drug screen, Nat. Rev. Cancer. 6 (2006) 813–823.
[32] L.Y. Frolova, Y.A. Meldrays, L.L. Kochkina, S.A. Giller, A.V. Eremeyev, N.A. Grayevskaya, L.L. Kisselev, DNA-polymerase inhibitors. Rifamycin derivatives, Nucleic Acids Res. 4 (3) (1977) 523–538.
[33] C. Seral, J.-M. Michot, H. Chanteux, M.-P. Mingeot-Leclercq, P.M. Tulkens, F. Van Bambeke, Influence of P-glycoprotein inhibitors on accumulation of macrolides in J774 murine macrophages, Antimicrob, Agents Chemother. 47 (2003) 1047–1051.
[34] A. Gvozdjakova, H. Ivanovicova, Synthesis and reactions of both tautomers of 2-hydrazinobenzothiazole, Chem. Pap. 40 (6) (1986) 797–800.
[35] P. Jimonet, F. Audiau, M. Barreau, J.-C. Blanchard, A. Boireau, Y. Bour, M.-A. Coléno, A. Doble, G. Doerflinger, C. Do Huu, M.-H. Donat, J.M. Duchesne, P. Ganil, C. Guérémy, E. Honoré, B. Just, R. Kerphirique, S. Gontier, P. Hubert, P.M. Laduron, J. Le Blevec, M. Meunier, J.-M. Miquet, C. Nemecek, M. Pasquet, O. Piot, J. Pratt, J. Rataud, M. Reibaud, J.-M. Stutzmann, S. Mignani, Riluzole Series. Synthesis and in vivo "antiglutamate" activity of 6-substituted-2-benzothiazolamines and 3-substituted-2-imino-benzothiazolines, J. Med. Chem. 42 (1999) 2828–2843.
[36] CrysAlisPro, Oxford Diffraction Ltd., Version 1.171.33.41c (release 11-05-2009 CrysAlis171.NET).
[37] G.M. Sheldrick, A short history of SHELX, Acta Cryst A64 (2009) 112–122.
[38] L.J. Farrugia, ORTEP-3 for Windows - a version of ORTEP-III with a Graphical User Interface (GUI), J. Appl. Cryst 30 (1997) 565.
[39] L.J. Farrugia, WinGX suite for small-molecule single-crystal crystallography, J. Appl. Cryst 32 (1999) 837–838.
[40] A.L. Spek, Single-crystal structure validation with the program PLATON, J. Appl. Cryst 36 (2003) 7–13.