European Journal of Medicinal Chemistry 44 (2009) 1396–1404

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis of novel thiazolone-based compounds containing pyrazoline moiety and evaluation of their anticancer activity

Dmytro Havrylyuk [a], Borys Zimenkovsky [a], Olexandr Vasylenko [c], Lucjusz Zaprutko [b], Andrzej Gzella [b], Roman Lesyk [a,*]

[a] Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Pekarska 69, Lviv 79010, Ukraine
[b] Department of Organic Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6, Poznan 60-780, Poland
[c] Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Science of Ukraine, Murmanska 1, Kyiv 02094, Ukraine

ARTICLE INFO

Article history:
Received 3 March 2008
Received in revised form 1 September 2008
Accepted 16 September 2008
Available online 7 October 2008

Keywords:
Thiazolones
Pyrazolines
Knoevenagel reaction
X-ray study
Anticancer activity

ABSTRACT

To examine the anticancer activity several novel thiazolone-based compounds containing 5-aryl-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl framework were obtained. Reaction of 5-aryl-3-phenyl-4,5-dihydropyrazole with 4-thioxo-2-thiazolidinone or 2-carbethoxymethylthio-2-thiazoline-4-one yielded starting 4- (**1** and **2**) or 2-substituted (**11** and **12**) thiazolones which were utilized in Knoevenagel condensation for obtaining a series of 5-arylidene derivatives **3**–**10**, **13**–**18**. Alternatively **11**, **12** and their 5-arylidene derivatives were synthesized by means of 3-phenyl-5-aryl-1-thiocarbamoyl-2-pyrazoline as S,N-binucleophile via [2 + 3]-cyclocondensation approach. The structures of compounds were determined by ^{1}H, ^{13}C NMR, LC–MS, EI-MS and X-ray analysis. The *in vitro* anticancer activity of synthesized compounds were tested by the National Cancer Institute and most of them displayed anticancer activity on leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancer cell lines. Relations between structure and activity are discussed, the most efficient anticancer compound **16** was found to be active with selective influence on colon cancer cell lines, especially on HT 29 ($\log GI_{50} = -6.37$).

1. Introduction

Thiazolidine template is one of the privileged structure fragments in modern medicinal chemistry considering its broad pharmacological spectrum and affinity for various biotargets of these class heterocyclic compounds [1]. Some of thiazolidine derivatives, especially 4-thiazolidinones, are PPAR-receptors agonists showing hypoglycemic, antineoplastic and anti-inflammatory activities [2], complex COX-2/5-LOX inhibitors [3] or phospholipase A_2 (PLA_2) [4] possessing anti-inflammatory action, and UDP-MurNAc/L-Ala-ligase inhibitors with antimicrobial effect [5]. Antineoplastic properties of 4-thiazolidinones and related heterocycles can most probably be caused by their affinity to anticancer biotargets, such as JNK-stimulating phosphatase-1 (JSP-1) [6], tumor necrosis factor TNFα [7], anti-apoptotic biocomplex Bcl-X_L-BH3 [8], integrin $\alpha_v\beta_3$ receptor [9], etc. It must be emphasized, that combination of thiazolidine template with other heterocycles is a well-known approach for drug-like molecules' build-up, which allows to achieve new pharmacological profile, action strengthening or toxicity lowering [1]. As part of our ongoing research in discovery of new active anticancer compounds [10–12] in this work we try to study the influence of pyrazoline moiety and thiazolone scaffold combination on the anticancer effect. The structural variations were explored by placing the pyrazoline moiety in positions 2 or 4 and introduction of different arylidene substituents in position 5 of thiazolone moiety (Fig. 1). The latter were recently exploited as bioactive arms on heterocyclic scaffolds useful to pharmacological effect realization of thiazolidinone based compounds.

2. Results and discussion

2.1. Chemistry

The general methods for synthesis of target pyrazoline substituted thiazolones are depicted in Schemes 1 and 2.

4-(5-Aryl-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-1,3-thiazol-2(5*H*)-ones **1** and **2** were obtained by means of 4-thioxo-2-thiazolidinone (isorhodanine) reaction with appropriate 5-aryl-3-phenyl-2-pyrazoline in refluxing ethanol (Scheme 1). Aiming at the detailed elaboration of structure–activity relationship isomeric 2-(5-aryl-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-1,3-thiazol-4(5*H*)-ones **11** and **12** were synthesized (Scheme 2). It should be noted, that 2-thioxo-4-thiazolidinone (rhodanine) usage for synthesis of target compounds failed. Therefore with the aim of increasing the

* Corresponding author. Tel.: +38 0322 75 59 66; fax: +38 0322 75 77 34.
E-mail address: dr_r_lesyk@org.lviv.net (R. Lesyk).

0223-5234/$ – see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.09.032

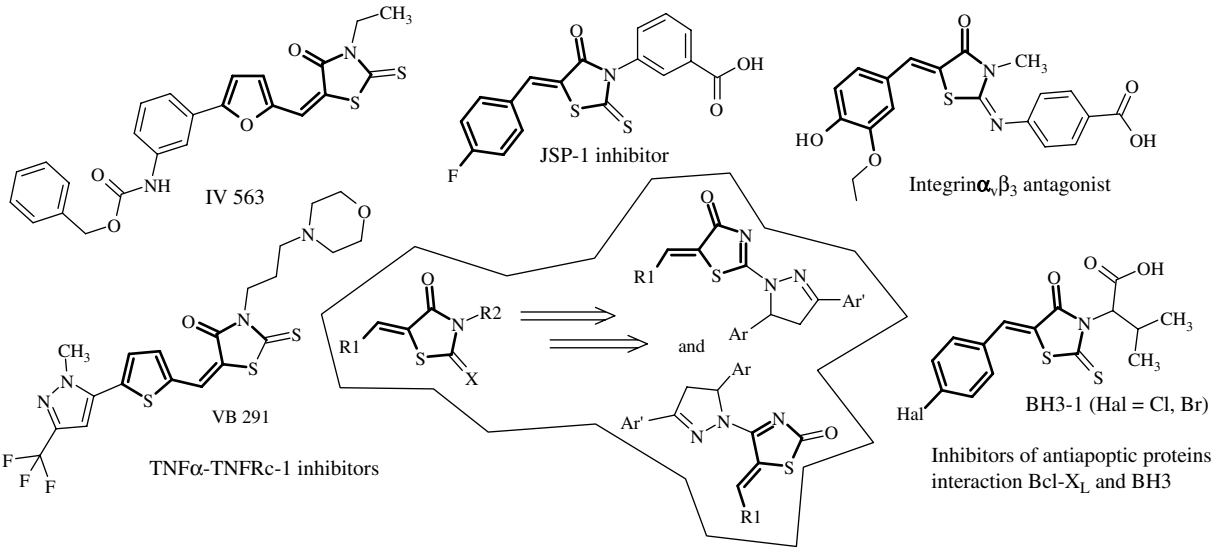


Fig. 1. Structures of anticancer 4-thiazolidinones and rationale for thiazolone-based compounds with pyrazoline moiety synthesis.

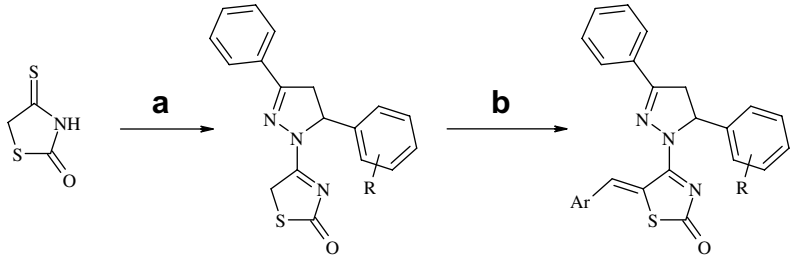
rhodanine reactivity, the latter was alkylated via intermediate triethylammonium salt by ethylchloroacetate in refluxing acetone, as reported [13]. Following reaction of 2-carbethoxymethylthio-2-thiazoline-4-one with appropriate 5-aryl-3-phenyl-2-pyrazolines in refluxing ethanol target 2-(5-aryl-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-1,3-thiazol-4(5*H*)-ones were obtained. Alternatively compounds **11** and **12** were synthesized following [2 + 3]-cyclo-condensation of 3-phenyl-5-aryl-1-thiocarbamoyl-2-pyrazolines with chloroacetic acid in the presence of fused sodium acetate in refluxing acetic acid.

Synthesized compounds **1**, **2** and **11**, **12** are methylene active heterocycles. On the other hand, it was previously established [1,13], that in most cases the presence and nature of moiety in position 5 of thiazolidinones play the key role in realization and character of pharmacological effects. The above mentioned thesis was rationale for synthesis of new 5-arylidene derivatives **3**–**10**, **13**–**18**, using standard Knoevenagel reaction procedure (medium – acetic acid, catalyst – fused sodium acetate) [1,13]. Some compounds (**11**, **12**, **14**, **17** and **18**) were prepared alternatively by one-pot methodology involving reaction of 3-phenyl-5-aryl-1-thiocarbamoyl-2-pyrazolines with chloroacetic acid and appropriate aromatic aldehydes in the presence of fused sodium acetate in refluxing acetic acid (Scheme 2).

The characterization data of synthesized novel pyrazoline substituted thiazolones are presented in experimental part. Analytical and spectral data (^{1}H NMR, ^{13}C NMR, LC–MS, EI-MS) confirmed the structure of the synthesized compounds.

Protons CH_2–CH of pyrazoline fragment in the ^{1}H NMR spectra of synthesized compounds show characteristic patterns of an AMX system. The chemical shifts of the protons H_A, H_M, and H_X have been assigned to about $\delta \sim 3.26$–3.45, $\delta \sim 3.96$–4.17, and $\delta \sim 5.68$–6.13, respectively, with corresponding coupling constants of $J_{AM} = 17.9$–18.6, $J_{Ax} = 10.4$–11.6, and $J_{Mx} = 2.9$–4.5 Hz. The chemical shift for the methylidene group of 5-arylidene derivatives **3**–**10** and **13**–**18** is insignificantly displaced in weak magnetic field, $\delta \sim 9.0$ and $\delta \sim 7.5$, respectively, and clearly indicated that only *Z*-isomers were obtained in Knoevenagel reaction of pyrazoline substituted thiazolones with aromatic aldehydes [15].

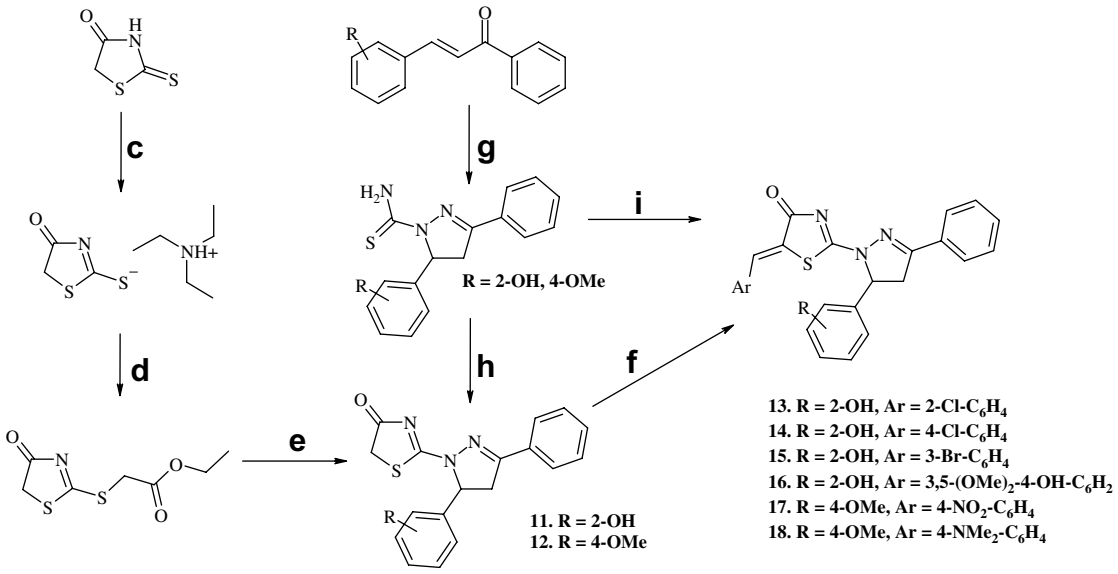
Structural features of synthesized 4-(5-aryl-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-1,3-thiazol-2(5*H*)-ones were confirmed by X-ray crystallographic analysis of exemplified compound **5**. As follows from the X-ray analysis the compound obtained has the structure of



1. R = 2-OH
2. R = 4-OMe

3. R = 2-OH, Ar = Ph
4. R = 2-OH, Ar = 4-Cl-C_6H_4
5. R = 2-OH, Ar = 4-Br-C_6H_4
6. R = 2-OH, Ar = 4-OH-C_6H_4
7. R = 2-OH, Ar = 4-OMe-C_6H_4
8. R = 4-OMe, Ar = 4-Cl-C_6H_4
9. R = 4-OMe, Ar = 4-NMe_2-C_6H_4
10. R = 4-OMe, Ar = 4-NO_2-C_6H_4

Scheme 1. Synthesis of novel 4-(5-aryl-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-1,3-thiazol-2(5*H*)-ones. Reagents, conditions and yields: (a) 5-(2-hydroxy or 4-methoxyphenyl)-3-phenyl-2-pyrazoline (1.0 equiv), EtOH, reflux 1 h, 62–67%; (b) Ar-CHO (1.1 equiv), AcONa (1.0 equiv), AcOH, reflux 2 h, 52–72%.



Scheme 2. Synthesis of novel 2-(5-aryl-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-1,3-thiazol-4(5*H*)-ones. Reagents, conditions and yields: (c) triethylamine (1.0 equiv), *i*-PrOH, rt 2 h, 88% (Ref. [13]); (d) $ClCH_2COOEt$ (1.0 equiv), acetone, reflux 2 h, 62% (Ref. [13]); (e) 5-(2-hydroxy or 4-methoxyphenyl)-3-phenyl-2-pyrazoline (1.0 equiv), EtOH, reflux 1 h, 72–79%; (f) Ar-CHO (1.2 equiv), AcONa (1.0 equiv), AcOH, reflux 5 h, 59–73%; (g) thiosemicarbazide (1.2 equiv), KOH (2.5 equiv), EtOH, reflux 8 h, 75–80% (Ref. [14]); (i) Ar-CHO (1.2 equiv), $ClCH_2COOH$ (1.0 equiv), AcONa (2.0 equiv), AcOH, reflux 5 h, 65–69%.

5-(4-bromobenzylidene)-4-[5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-2(5*H*)-one (**5**) (Scheme 1, Fig. 2). The interatomic distances N3–C4 [1.313(4) Å] and C4–N7 [1.341(4) Å], longer than the normal length of the double bond C=N, 1.279(1) Å [16] only by about 8 and 15σ, indicate the partly double character of the two bonds. This suggests that in the solid state the compound analyzed has a structure intermediate between that of **5a** and that of **5b** (Scheme 3).

The bond lengths 1.342(4) Å and 1.289(3) Å confirm the occurrence of the double bonds between C5, C25 and N8, C9 atoms, respectively (Fig. 3).

The five-membered dihydropyrazole ring has an envelope conformation, with puckering parameters [17] of $Q = 0.176(3)$ Å, $\varphi = 319.1(9)°$. The deviation of C11 atom from the almost planar system of the other four atoms of the heterocyclic ring is 0.286(4) Å.

The conformation of the molecule of compound **5** is stabilized by the intra- and intermolecular interactions. The intramolecular hydrogen bond C31–H···S1 [C31···S1, 3.136(4) Å] favours the arrangement of the *p*-bromobenzylidene and thiazolidine systems in the molecule at the angle of 24.44(13)°, while the hydrogen bond C25–H···N8 [C25···N8, 2.910(4) Å] stabilizes the almost coplanar arrangement of the dihydropyrazole and thiazolidine rings. The dihedral angle made by the rings is only 9.26(13)°. The significant deviation of the hydroxyphenyl ring from the least squares plane of the dihydropyrazole ring of 74.88(10)° is favoured by the intermolecular hydrogen bond O24–H24···O33 [O24···O33, 2.656(4) Å], made by the hydroxyl group of the hydroxyphenolic system and the carbonyl oxygen atom of the molecule of the crystallization solvent (dimethylformamide) involved in the formation of the crystal lattice of **5**.

2.2. *Evaluation of anticancer activity* in vitro

Some new pyrazoline substituted thiazolones (**1**, **6**, **11** and **13**–**16**) were submitted and evaluated at single concentration of 10^{-5} M towards panel of approximately sixty cancer cell lines. The human tumor cell lines were derived from 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 [18–22]. The compounds were added at a 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 of treated cells when compared to untreated control cells (Table 1).

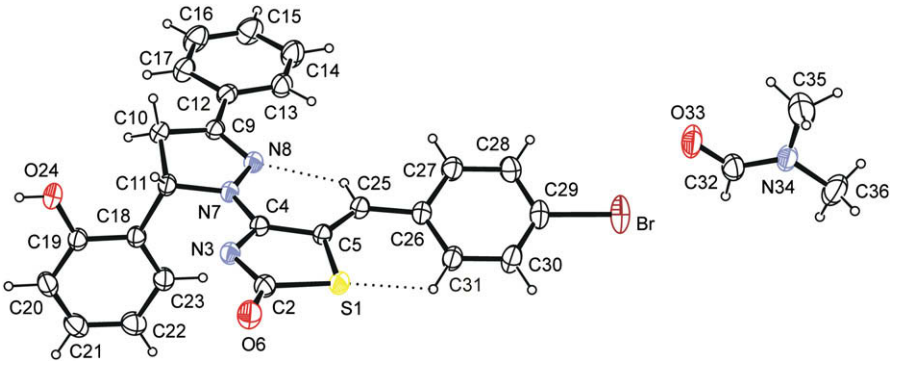
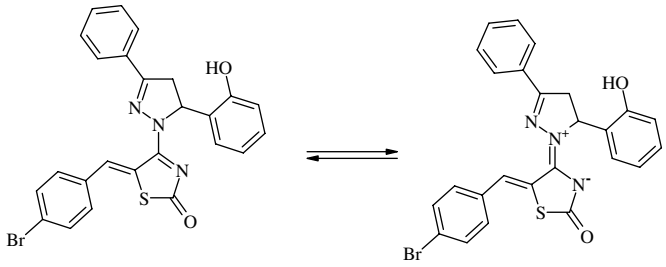


Fig. 2. The molecular structure of **5** with displacement ellipsoids drawn at the 30% probability level. H atoms, treated as isotropic, are on an arbitrary scale.



Form a **Form b**

Scheme 3. Two mesomeric forms of **5**.

Range of growth % shows the lowest and the highest growth % found among different cancer cell lines.

Finally, compounds **6** and **16** possessed considerable activity and were selected, whereas compounds **3**, **4**, **7** and **17** were tested without preliminary pre-screening stage, in advanced assay against a panel of approximately sixty tumor cell lines at 10-fold dilutions of five concentrations (100, 10, 1, 0.1 and 0.01 μM) [18–22]. The percentage of growth was evaluated spectrophotometrically versus controls not treated with test agents. A 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 antitumor 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 a 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, insensitive cell lines are included with the highest concentration tested (Table 2).

The tested compounds showed a broad spectrum of growth inhibition activity against human tumor cells, as well as some distinctive patterns of selectivity. Compounds **3**, **6** and **16** showed the highest cytotoxicity and were active against all tested human tumor cell lines (Table 2). Selectivity pattern analysis of cell lines by disease origin can definitely affirm selective action of compounds **3** and **6** on leukemia cell lines and compound **16** on colon cancer (Fig. 4). These compounds appeared to be the most active against selected individual cell lines with the log GI_{50} varying from −6.37 to −5.70 (Table 3). Compound **3** was found to be a highly active growth inhibitor of the leukemia cell line RPMI-8226, non-small cell lung cancer cell line HOR-92, colon cancer line HCT 15 and CNS cancer cell line U251. Compound **6** showed selectivity on leukemia cell lines (HL-60(TB) and RPMI-8226), colon cancer line HT-29, CNS cancer line SNB-75 and melanoma (SK-MEL-28, SK-MEL-5 and UACC-62). Pyrazoline substituted derivative **16** demonstrates the most marked effect among all synthesized compounds and possessed significant activity on non-small cell lung cancer cell line HOP-62 and colon cancer cell line HT-29 with log GI_{50} value −6.12 and −6.37, respectively.

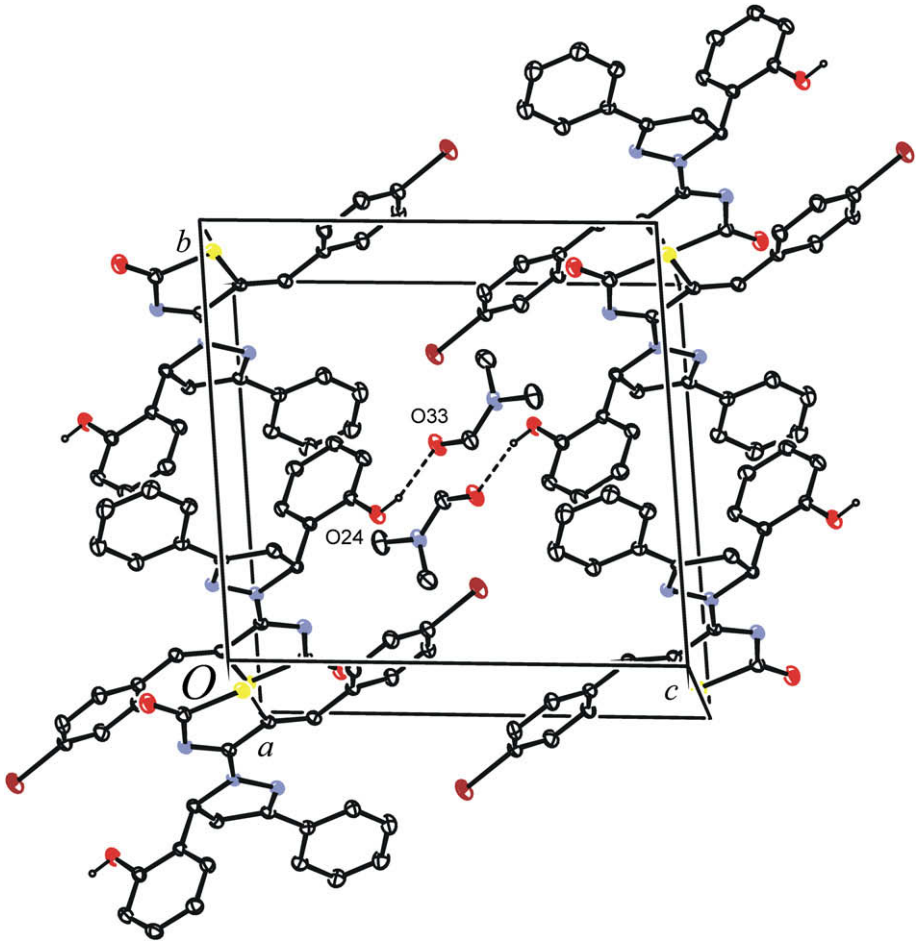


Fig. 3. Molecular packing and hydrogen bonds (dashed lines); H atoms not involved in hydrogen bonds have been omitted for clarity.

Table 1
Anticancer screening data in concentration 10^{-5} M.

Compound	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	
1	83.59	−5.39 to 124.97	CCRF-CEM (Leukemia)	−5.39	Inactive
6	1.13	−96.31 to 134.28	SK-MEL-5 (Melanoma)	−96.31	Active
11	101.04	−61.48 to 145.90	MOLT-4 (Leukemia)	−61.48	Inactive
13	102.39	45.62 to 158.52	SR (Leukemia)	45.62	Inactive
14	98.33	−25.00 to 161.62	CCRF-CEM (Leukemia)	−25.00	Inactive
15	88.58	−53.95 to 164.31	HL-60(TB) (Leukemia)	−53.95	Inactive
16	42.59	−57.04 to 123.35	DU-145 (Prostate cancer)	−57.04	Active

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 [18,22] analyses were performed for active compounds at GI_{50} level, however, obtained correlation coefficients (r) didn't allow to distinguish cytotoxicity mechanism of tested compounds with high probability. Nevertheless moderate correlations compound **4** showed with dichloroallyl lawsone (NSC 126771, dihydroorotate dehydrogenase inhibitor, $r = 0.600$ [23]), as well as compound **6** with tritylcysteine (NSC 83265, aminoacyl-tRNA synthetases inhibitor with antiproliferative effect against leukemia, $r = 0.537$ [24]).

The SAR study revealed that: (1) anticancer activity of compounds **1**, **3**, **4**, **6**, **7**, **11** and **13**–**17** is sensitive to the nature of substituent in position 5 of thiazolone cycle, (2) introduction of *p*-OH group in 5-benzylidene fragment enhanced potency, and (3) linking position of pyrazoline fragment (2 or 4) with thiazolone core did not influence antitumor activity.

3. Conclusions

In the present paper, eighteen new pyrazoline substituted thiazolones were described. Eleven of synthesized compounds were tested and most of them displayed antitumor activity on 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 **3**, **6** and **16**, especially 5-(4-hydroxy-3,5-dimethoxybenzylidene)-2-[5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]thiazol-4(5*H*)-one (**16**) could be prospective antitumor agent (average log GI_{50} and log TGI values −5.61 and −5.14, respectively) with selective influence on colon cancer cell lines. The obtained results prove the necessity for further investigations to clarify the features underlying the antitumor potential of tested compounds.

4. Experimental

4.1. Materials and methods

The starting 2-thioxo-4-thiazolidinone [25], 4-thioxo-2-thiazolidinone [26], 5-aryl-3-phenyl-2-pyrazolines [27], 3-phenyl-5-aryl-1-thiocarbamoyl-2-pyrazolines [14], 2-carbethoxymethylthio-2-thiazoline-4-one [13] 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 the 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. LC–MS and EI-MS were obtained on Agilent 1100 and Varian 1200L instruments, respectively.

4.2. Chemistry

4.2.1. General procedure for synthesis of 4-(5-aryl-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-1,3-thiazol-2(5H)-ones (*1* and *2*)

A mixtures of 4-thioxo-2-thiazolidinone (20 mmol) and appropriate 5-aryl-3-phenyl-2-pyrazoline (20 mmol) were 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 *n*-butanol (**1**) or DMF:ethanol, 1:2 mixture (**2**).

Table 2
Summary of anticancer screening data at dose-dependent assay.

Compound	*N*[a]	log GI_{50}			log TGI			log LC_{50}		
		*N*1[b]	Range	MG_MID	*N*2[b]	Range	MG_MID	*N*3[b]	Range	MG_MID
3	54	54	−5.82 to −4.43	−5.41	52	−5.54 to −4.00	−4.90	37	−5.54 to −4.00	−4.38
4	56	56	−5.73 to −4.22	−4.97	55	−5.45 to −4.00	−4.57	49	−5.16 to −4.00	−4.21
6	57	57	−5.78 to −4.59	−5.46	23	−5.48 to −4.00	−4.45	5	−4.31 to −4.00	−4.02
7	57	54	−5.08 to −4.00	−4.55	34	−4.60 to −4.00	−4.18	11	−4.30 to −4.00	−4.04
16	57	57	−6.37 to −4.74	−5.61	55	−5.53 to −4.37	−5.14	45	−5.27 to −4.00	−4.58
17	57	7	−5.28 to −4.00	−4.08	2	−4.36 to −4.00	−4.01	0	–	−4.00

[a] *N* – number of human tumor cell lines tested at the 2nd stage assay.
[b] *N*1, *N*2, *N*3 – number of sensitive cell lines, against which the compound possessed considerable growth inhibition according to mentioned parameter (parameters log GI_{50}, log TGI and log $LC_{50} \leq -4.00$).

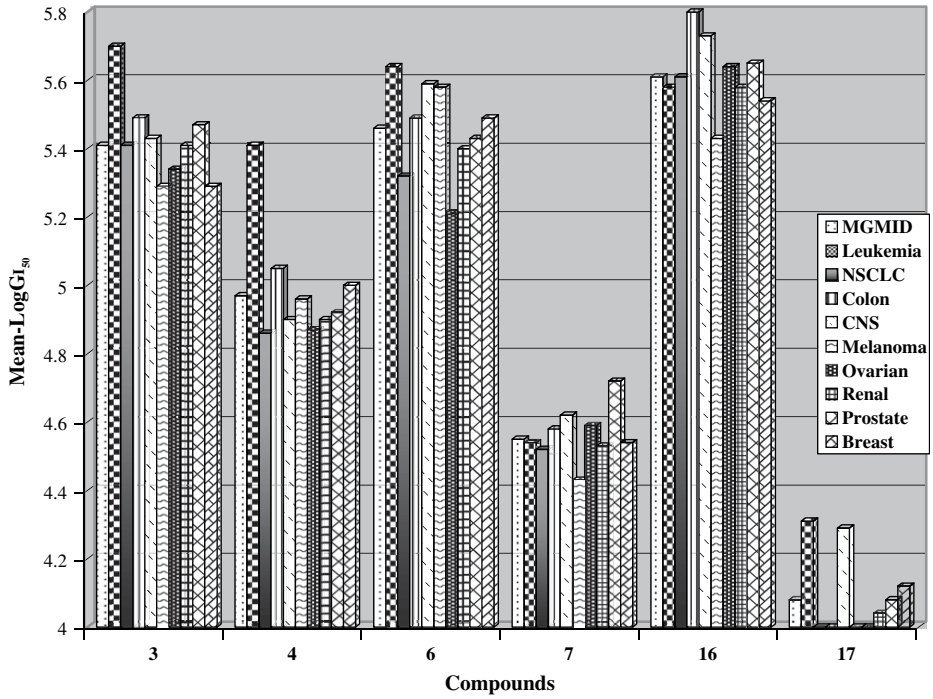


Fig. 4. Anticancer selectivity pattern of the most active compounds **3**, **4**, **6**, **7**, **16** and **17**.

*4.2.1.1. 4-[5-(2-Hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-2(5*H*)-one (**1**).* Yield 67%, mp 181–183 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4): δ 9.65 (s, 1H), 7.86 (d, J = 8.8 Hz, 2H), 7.52–7.43 (m, 3H), 7.10 (t, J = 8.2 Hz, 1H), 6.98 (d, J = 8.0 Hz, 1H), 6.88 (d, J = 8.0 Hz, 1H), 6.75 (t, J = 8.2 Hz, 1H), 5.95 (dd, J = 10.6, 4.0 Hz, 1H), 4.70 (d, J = 18.2 Hz, 1H), 4.58 (d, J = 18.2 Hz, 1H), 4.00 (dd, J = 18.4, 10.6 Hz, 1H), 3.35 (dd, J = 18.4, 4.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.2 (C=O), 175.4 (C=N, thiaz.), 161.7 (C=N, pyraz.), 154.9 (C–OH), 134.0, 132.0, 130.8, 129.2, 128.9, 127.9, 127.3, 119.6, 116.4, 59.2 ($\underline{C}HCH_2$), 42.6 ($CH\underline{C}H_2$), 38.5 (CH_2, thiaz.). Anal. $C_{18}H_{15}N_3O_2S$ (C, H, $\overline{N}$).

*4.2.1.2. 4-[5-(4-Methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-2(5*H*)-one (**2**).* Yield 62%, mp 237–238 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4): δ 7.85 (d, J = 8.1 Hz, 2H), 7.53–7.46 (m, 3H), 7.17 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 5.68 (dd, J = 11.1, 3.9 Hz, 1H), 4.75 (d, J = 18.1 Hz, 1H), 4.62 (d, J = 18.1 Hz, 1H), 4.07 (dd, J = 18.3, 11.1 Hz, 1H), 3.73 (s, 3H), 3.35 (dd, J = 18.3, 3.9 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.2 (C=O), 175.5 (C=N, thiaz.), 161.3 (C=N, pyraz.), 159.5 (C-OH), 132.9, 132.1, 130.7, 129.6, 128.1, 127.8, 114.8, 63.5 ($\underline{C}HCH_2$), 55.8 (OCH_3), 43.7 ($CH\underline{C}H_2$), 38.6 (CH_2, thiaz.). Anal. $C_{19}H_{17}\overline{N}_3O_2S$ (C, H, N).

*4.2.2. General procedure for synthesis of 5-arylidene-4-(5-aryl-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-1,3-thiazol-2(5*H*)-ones (**3**–**10**)*

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

*4.2.2.1. 5-Benzylidene-4-[5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-2(5*H*)-one (**3**).* Yield 63%, mp 204–206 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4): δ 9.80 (s, 1H), 9.20 (s, 1H), 7.86 (d, J = 8.8 Hz, 2H), 7.65 (d, J = 8.8 Hz, 2H), 7.54–7.46 (m, 5H), 7.40 (t, J = 8.8 Hz, 1H), 7.07 (t, J = 8.7 Hz, 1H), 6.90 (d, J = 8.7 Hz, 1H), 6.87 (d, J = 8.0 Hz, 1H), 6.70 (t, J = 8.2 Hz, 1H), 6.13 (dd, J = 10.6, 3.4 Hz, 1H), 3.94 (dd, J = 18.2, 10.6 Hz, 1H), 3.26 (dd, J = 18.2, 3.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.8 (C=O), 166.6 (C=N, thiaz.), 164.4 (C=N, pyraz.), 154.9 (C–OH), 137.7, 135.8, 132.4, 130.9, 129.9, 129.4, 129.2, 128.3, 127.3, 126.5, 119.7, 116.4, 63.7, ($\underline{C}HCH_2$), 40.9 ($CH\underline{C}H_2$). LC–MS: m/z 426 (95.96%, $M^+ + 1$). Anal. $C_{25}H_{19}N_3O_2S$ (C, H, $\overline{N}$).

*4.2.2.2. 5-(4-Chlorobenzylidene)-4-[5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-2(5*H*)-one (**4**).* Yield 64%, mp 225–227 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4): δ 9.80 (s, 1H), 9.10 (s, 1H), 7.88 (d, J = 8.8 Hz, 2H), 7.65 (d, J = 9.0 Hz, 2H), 7.48 (d, J = 9.0 Hz, 2H), 7.55–7.45 (m, 3H), 7.07 (t, J = 8.7 Hz, 1H), 6.94 (d, J = 8.7 Hz, 1H), 6.86 (d, J = 8.0 Hz, 1H), 6.74 (t, J = 8.2 Hz, 1H), 6.10 (dd, J = 10.4, 4.0 Hz, 1H), 3.96 (dd, J = 18.4, 10.4 Hz, 1H), 3.26 (dd, J = 18.4, 4.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.4 (C=O), 166.4 (C=N, thiaz.), 164.5 (C=N, pyraz.), 154.9 (C–OH), 136.1, 135.4, 134.6, 132.5, 129.8, 129.4, 128.9, 128.4, 126.8, 119.6, 116.4 (3-$\underline{C}$H 2-OH), 63.8 ($\underline{C}HCH_2$), 41.8 ($CH\underline{C}H_2$). LC–MS: m/z 460 (94.35%, $M^+ + 1$; 3.17%, $M^+ + 2$). Anal. $C_{25}H_{18}\overline{Cl}N_3O_2S$ (C, H, N).

*4.2.2.3. 5-(4-Bromobenzylidene)-4-[5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-2(5*H*)-one (**5**).* Yield 52%, mp 212–215 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4): δ 9.79 (s, 1H), 9.06 (s, 1H), 7.91 (d, J = 8.8 Hz, 2H), 7.68 (d, J = 8.8 Hz, 2H), 7.62 (d, J = 8.8 Hz, 2H), 7.56–7.48 (m, 3H), 7.11 (t, J = 8.7 Hz, 1H), 6.94 (d, J = 8.7 Hz, 1H), 6.88 (d, J = 8.0 Hz, 1H), 6.72 (t, J = 8.2 Hz, 1H), 6.07 (dd, J = 10.6, 3.2 Hz, 1H), 3.98 (dd, J = 18.2, 10.6 Hz, 1H), 3.26 (dd, J = 18.4, 3.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.4 (C=O), 166.5 (C=N, thiaz.), 164.6 (C=N, pyraz.), 154.9 (C–OH), 137.9, 132.9, 132.7, 132.6, 132.4, 130.1, 129.8, 129.4, 128.9, 128.4, 128.1, 126.8, 124.4, 122.4, 119.7, 116.4, 63.8 ($\underline{C}HCH_2$), 41.8 ($CH\underline{C}H_2$). Anal. $C_{25}H_{18}BrN_3O_2S$ (C, H, N).

*4.2.2.4. 5-(4-Hydroxybenzylidene)-4-[5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-2(5*H*)-one (**6**).* Yield 64%, mp 266–267 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4): δ 10.27 (s, 1H), 9.82 (s, 1H), 9.07 (s, 1H), 7.92 (d, J = 8.8 Hz, 2H), 7.60 (d, J = 8.8 Hz, 2H), 7.60–7.50 (m, 3H), 6.98 (d, J = 8.8 Hz, 2H), 7.08 (t, J = 8.7 Hz,

Table 3
The influence of compounds **3**, **4**, **6** and **16** on the growth of individual tumor cell lines (log $GI_{50} \leq -5.70$).

Compound	Disease	Cell line	log GI_{50}	log TGI
3	Leukemia	CCRF-CEM	−5.72	−5.34
	Leukemia	RPMI-8226	−5.81	−5.49
	NSC lung cancer	HOP-62	−5.79	−5.46
	NSC lung cancer	HOP-92	−5.81	−5.35
	Colon cancer	HCT-15	−5.82	−5.53
	Colon cancer	KM-12	−5.79	−5.35
	CNS cancer	U251	−5.81	−5.54
	Melanoma	UACC-62	−5.79	−5.53
	Ovarian cancer	OVCAR-3	−5.70	−5.35
	Renal cancer	786-0	−5.74	−5.41
	Renal cancer	SN12C	−5.77	−5.51
	Breast cancer	NCI/ADR-RES	−5.74	−5.42
4	Melanoma	LOX IMVI	−5.73	−5.45
6	Leukemia	HL-60(TB)	−5.78	−5.31
	Leukemia	RPMI-8226	−5.73	−5.35
	Colon cancer	HT-29	−5.72	–
	CNS cancer	SNB-75	−5.71	−5.28
	Melanoma	SK-MEL-28	−5.75	–
	Melanoma	SK-MEL-5	−5.77	−5.48
	Melanoma	UACC-62	−5.77	−5.48
16	Leukemia	CCRF-CEM	−5.70	−5.25
	Leukemia	MOLT-4	−5.77	–
	Leukemia	RPMI-8226	−5.77	−5.16
	NSC lung cancer	HOP-62	−6.12	−4.81
	NSC lung cancer	NCI-H460	−5.79	−5.48
	Colon cancer	HCT-116	−5.81	−5.53
	Colon cancer	HCT-15	−5.91	−5.57
	Colon cancer	HT29	−6.37	−5.55
	Colon cancer	KM12	−5.74	−5.44
	CNS cancer	SF-268	−5.73	−5.35
	CNS cancer	SNB-19	−5.75	−5.43
	CNS cancer	SNB-75	−5.76	−5.37
	CNS cancer	U251	−5.82	−5.55
	Melanoma	LOX IMVI	−5.81	−5.53
	Melanoma	MALME-3M	−5.70	−5.31
	Melanoma	SK-MEL-5	−5.72	−4.98
	Ovarian cancer	IGROV-1	−5.80	−5.54
	Ovarian cancer	OVCAR-3	−5.85	−5.55
	Renal cancer	ACHN	−5.93	−4.99
	Renal cancer	CAKI-1	−5.99	−5.49
	Renal cancer	SN12C	−5.80	−5.31
	Renal cancer	TK-10	−5.75	−5.40
	Renal cancer	UO-31	−5.72	−5.37
	Prostate cancer	DU-145	−5.81	−5.51
	Breast cancer	MCF7	−5.71	−5.31
	Breast cancer	MDA-MB-231/ATCC	−5.84	−5.52
	Breast cancer	HS 578T	−5.86	−5.43
	Breast cancer	MDA-MB-435	−5.74	−5.44
	Breast cancer	T-47D	−5.75	−5.40

1H), 6.93 (d, J = 8.7 Hz, 1H), 6.86 (d, J = 8.0 Hz, 1H), 6.74 (t, J = 8.2 Hz, 1H), 6.00 (dd, J = 11.4, 3.8 Hz, 1H), 3.99 (dd, J = 18.3, 11.4 Hz, 1H), 3.28 (dd, J = 18.3, 3.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.1 (C=O), 166.8 (C=N, thiaz.), 163.6 (C=N, pyraz.), 160.5 (C–OH-*p*), 154.9 (C–OH-*o*), 138.2, 133.4, 132.2, 130.9, 129.8, 129.3, 128.2, 127.0, 126.8, 126.4, 124.6, 119.7, 116.9, 116.4, 63.5 ($\underline{C}HCH_2$), 36.5 ($CH\underline{C}H_2$). Anal. $C_{25}H_{19}N_3O_3S$ (C, H, N).

*4.2.2.5. 5-(4-Methoxybenzylidene)-4-[5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-2(5*H*)-one (**7**).* Yield 72%, mp 256–257 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4): δ 9.60 (s, 1H), 9.08 (s, 1H), 7.88 (d, J = 8.8 Hz, 2H), 7.62 (d, J = 8.6 Hz, 2H), 7.53–7.43 (m, 3H), 7.04 (d, J = 8.6 Hz, 2H), 7.08 (t, J = 8.7 Hz, 1H), 6.93 (d, J = 8.7 Hz, 1H), 6.88 (d, J = 8.0 Hz, 1H), 6.72 (t, J = 8.2 Hz, 1H), 6.11 (dd, J = 10.6, 3.6 Hz, 1H), 3.92 (dd, J = 18.2, 10.6 Hz, 1H), 3.88 (s, 3H), 3.28 (dd, J = 18.2, 3.6 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.9 (C=O), 166.7 (C=N, thiaz.), 163.8 (C=N, pyraz.), 161.5 (CH_3–O), 154.9 (C–OH), 137.7, 133.0, 132.3, 130.8, 129.8, 129.4, 128.2, 127.9, 127.1, 126.8, 125.9, 119.7 (5-$\underline{C}H$, 2-OH), 116.4, 115.5, 63.6 ($\underline{C}HCH_2$), 56.2 (CH_3–O), 40.9 ($CH\underline{C}H_2$). Anal. $C_{26}H_{21}N_3O_3S$ (C, H, N).

*4.2.2.6. 5-(4-Chlorobenzylidene)-4-[5-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-2(5*H*)-one (**8**).* Yield 66%, mp 231–232 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4): δ 9.03 (s, 1H), 7.96 (d, J = 7.2 Hz, 2H), 7.72 (d, J = 8.6 Hz, 2H), 7.60 (d, J = 8.6 Hz, 2H), 7.58–7.50 (m, 3H), 7.20 (d, J = 8.6 Hz, 2H), 6.90 (d, J = 8.6 Hz, 2H), 5.92 (dd, J = 10.8, 3.1 Hz, 1H), 4.01 (dd, J = 18.6, 10.8 Hz, 1H), 3.88 (s, 3H), 3.35 (dd, J = 18.2, 3.1 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6,): δ 176.4 (C=O), 166.6 (C=N, thiaz.), 164.1 (C=N, pyraz.), 159.4 (C–OH), 136.2, 135.4, 134.6, 133.1, 132.5, 130.6, 130.0, 129.9, 128.5, 127.8, 114.9, 66.4 ($\underline{C}HCH_2$), 55.8 (O–CH_3), 41.9 ($CH\underline{C}H_2$). EI-MS (m/z): 473 (M^+), 475 ($M^+ + 2$). Anal. $C_{26}H_{20}ClN_3O_2S$ (C, H, N).

*4.2.2.7. 5-(4-Dimethylaminobenzylidene)-4-[5-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-2(5*H*)-one (**9**).* Yield 57%, mp 233–234 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4): δ 9.01 (s, 1H), 7.92 (d, J = 7.2 Hz, 2H), 7.60 (d, J = 8.6 Hz, 2H), 7.58–7.50 (m, 3H), 7.46 (d, J = 8.6 Hz, 2H), 7.17 (d, J = 8.6 Hz, 2H), 6.84 (d, J = 8.6 Hz, 2H), 5.88 (dd, J = 11.0, 2.9 Hz, 1H), 3.96 (dd, J = 18.4, 11.0 Hz, 1H), 3.70 (s, 3H), 3.35 (dd, J = 18.4, 2.9 Hz, 1H), 3.02 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.3 (C=O), 166.7 (C=N, thiaz.), 162.3 (C=N, pyraz.), 159.4 (C–OH), 152.1 (4-NMe_2), 133.7, 133.6, 133.4, 132.8, 132.2, 130.9, 129.8, 128.1, 127.7, 122.2, 121.1, 114.8, 112.7 (3-$\underline{C}H$, 4-NMe_2), 66.1 ($\underline{C}HCH_2$), 55.8 (O-CH_3), 41.7 ($CH\underline{C}H_2$). EI-MS (m/z): 482 (M^+). Anal. $C_{28}H_{24}N_4O_2S$ (C, H, N).

*4.2.2.8. 5-(4-Nitrobenzylidene)-4-[5-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-2(5*H*)-one (**10**).* Yield 68%, mp 239–240 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4): δ 9.10 (s, 1H), 8.34 (d, J = 8.8 Hz, 2H), 7.95 (d, J = 8.8 Hz, 2H), 7.93 (d, J = 8.8 Hz, 2H), 7.58–7.54 (m, 3H), 7.23 (d, J = 8.7 Hz, 2H), 6.89 (d, J = 8.7 Hz, 2H), 5.94 (dd, J = 11.0, 3.5 Hz, 1H), 4.03 (dd, J = 18.4, 11.0 Hz, 1H), 3.74 (s, 3H), 3.34 (dd, J = 18.4, 3.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.9 (C=O), 166.4 (C=N, thiaz.), 164.7 (C=N, pyraz.), 159.5 (C–O), 147.9, 142.1, 134.9, 133.6, 132.9, 132.7, 131.7, 130.4, 129.8, 128.6, 127.9, 124.7, 114.9, 66.6 ($\underline{C}HCH_2$), 55.8 (O–CH_3), 41.9 ($CH\underline{C}H_2$). Anal. $C_{26}H_{20}N_4O_4S$ (C, H, N).

*4.2.3. General procedure for synthesis of 2-(5-aryl-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-1,3-thiazol-4(5*H*)-ones (**11** and **12**)*

Method A. A mixture of 2-carbethoxymethylthio-2-thiazoline-4-one (20 mmol) and appropriate 5-aryl-3-phenyl-2-pyrazoline (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 DMF:ethanol, 1:2 mixture.

Method B. A mixture of 3-phenyl-5-aryl-1-thiocarbamoyl-2-pyrazoline (10 mmol), chloroacetic acid (10 mmol) and anhydrous sodium acetate (10 mmol) was refluxed for 3 h in glacial acetic acid (10 ml). Obtained powder was filtered off, washed with methanol and recrystallized with DMF:ethanol, 1:2 mixture.

*4.2.3.1. 2-[5-(2-Hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**11**).* Yield 79% (method A), 68% (method B), mp 255–256 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4): δ 9.70 (s, 1H), 7.81 (d, J = 7.4 Hz, 2H), 7.45–7.42 (m, 3H), 7.06 (t, J = 7.6 Hz, 1H), 6.87 (d, J = 7.6 Hz, 1H), 6.84 (d, J = 7.6 Hz, 1H), 6.71 (t, J = 7.6 Hz, 1H), 5.87 (dd, J = 11.3, 4.2 Hz, 1H), 4.01 (dd, J = 18.2, 11.3 Hz, 1H), 3.79 (s, 2H), 3.30 (dd, J = 18.2, 4.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 187.5 (C=O), 177.8 (C=N, thiaz.), 161.5 (C=N, pyraz.), 154.9 (C–OH), 132.0, 130.7, 129.6, 129.5, 127.9, 127.5, 126.5, 119.7, 116.4, 61.4 ($\underline{C}HCH_2$), 42.8 ($CH\underline{C}H_2$), 39.4 (CH_2). Anal. $C_{18}H_{15}N_3O_2S$ (C, H, N).

*4.2.3.2. 2-[5-(4-Methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**12**).* Yield 72% (method A), 61% (method B), mp 135–137 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4): δ 7.83 (d, J = 7.3 Hz, 2H), 7.53–7.48 (m, 3H), 7.15 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 5.72 (dd, J = 11.3, 3.8 Hz, 1H), 4.06 (dd, J = 18.3, 11.3 Hz, 1H), 3,84 (s, 2H), 3.73 (s, 3H), 3.36 (dd, J = 18.3, 3.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 187.5 (C=O), 177.9 (C=N, thiaz.), 161.2 (C=N, pyraz.), 159.4 (C–OH), 133.1, 132.2, 130.5, 129.7, 128.0, 127.8, 114.9, 63.9 (CHCH$_2$), 55.8 (O–CH$_3$), 44.0 (CHCH$_2$), 40.4 (CH$_2$). Anal. $C_{19}H_{17}N_3O_2S$ (C, H, N).

*4.2.4. General procedure for synthesis of 5-arylidene-2-(5-aryl-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-1,3-thiazol-4(5*H*)-ones (**13**–**18**)*

Method A. A mixture of compounds **11** or **12** (3 mmol), appropriate aldehyde (4 mmol) and anhydrous sodium acetate (3 mmol) was refluxed for 5 h in glacial acetic acid (30 ml). Powder obtained after cooling was filtered off, washed with methanol and recrystallized with DMF:ethanol or DMF:acetic acid, 1:2 mixtures.

Method B. A mixture of 3-phenyl-5-aryl-1-thiocarbamoyl-2-pyrazoline (10 mmol), chloroacetic acid (10 mmol), appropriate aldehyde (12 mmol) and anhydrous sodium acetate (10 mmol) was refluxed for 5 h in glacial acetic acid (10 ml). Powder obtained after cooling was filtered off, washed with methanol and recrystallized with DMF:ethanol or DMF:acetic acid, 1:2 mixtures.

*4.2.4.1. 5-(2-Chlorobenzylidene)-2-[5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**13**).* Yield 59% (method A), mp 268–269 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4): δ 9.75 (s, 1H), 7.87 (d, J = 7.4 Hz, 2H), 7.85 (s, 1H), 7.70 (d, J = 7.3 Hz, 1H), 7.52–7.42 (m, 5H), 7.39 (t, J = 7.5 Hz, 1H), 7.09 (t, J = 7.1 Hz, 1H), 6.93 (d, J = 7.5 Hz, 1H), 6.87 (d, J = 8.0 Hz, 1H), 6.73 (t, J = 7.4 Hz, 1H), 5.94 (dd, J = 11.4, 4.1 Hz 1H), 4.08 (dd, J = 18.0, 11.4 Hz, 1H), 3.38 (dd, J = 18.0, 4.1 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 179.3 (C=O), 170.6 (C=N, thiaz.), 162.9 (C=N, pyraz.), 155.1 (C–OH), 134.9, 132.7, 132.5, 132.4, 131.9, 130.9, 130.4, 129.8, 129.7, 129.6, 128.8, 128.1, 126.3, 125.9, 119.7, 116.4, 61.8 (CHCH$_2$), 42.9 (CHCH$_2$). Anal. $C_{25}H_{18}ClN_3O_2S$ (C, H, N).

*4.2.4.2. 5-(4-Chlorobenzylidene)-2-[5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**14**).* Yield 68% (method A), 69% (method B), mp 284–286 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4): δ 9.74 (s, 1H), 7.88 (d, J = 8.0 Hz, 2H), 7.60 (d, J = 8.5 Hz, 2H), 7.57 (s, 1H), 7.50–7.42 (m, 5H), 7.08 (t, J = 7.5 Hz, 1H), 6.92 (d, J = 8.0 Hz, 1H), 6.86 (d, J = 8.0 Hz, 1H), 6.73 (t, J = 7.4 Hz, 1H), 5.97 (dd, J = 11.4, 4.5 Hz, 1H), 4.08 (dd, J = 18.1, 11.4 Hz, 1H), 3.38 (dd, J = 18.1, 4.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 179.7 (C=O), 170.4 (C=N, thiaz.), 162.6 (C=N, pyraz.), 155.1 (C–OH), 135.0, 133.5, 132.3, 131.9, 130.4, 129.9, 129.8, 129.7, 129.6, 128.1, 126.0, 119.7, 116.4, 61.7 (CHCH$_2$), 42.9 (CHCH$_2$). Anal. $C_{25}H_{18}ClN_3O_2S$ (C, H, N).

*4.2.4.3. 5-(3-Bromobenzylidene)-2-[5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**15**).* Yield 62% (method A), mp 272–274 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4): δ 9.84 (s, 1H), 7.89 (d, J = 7.9 Hz, 2H), 7.74 (s, 1H), 7.59 (d, J = 8.6 Hz, 1H), 7.56–7.40 (m, 6H), 7.08 (t, J = 8.2 Hz, 1H), 6.92 (d, J = 8.2 Hz, 1H), 6.86 (d, J = 8.2 Hz, 1H), 6.72 (t, J = 8.2 Hz, 1H), 5.98 (dd, J = 11.3, 4.5 Hz, 1H), 4.08 (dd, J = 18.4, 11.3 Hz, 1H), 3.38 (dd, J = 18.4, 4.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 179.5 (C=O), 170.4 (C=N, thiaz.), 162.8 (C=N, pyraz.), 155.1 (C–OH), 137.1, 133.1, 132.4, 131.9, 130.5, 130.4, 129.7, 128.6, 128.1, 126.0, 123.1, 119.7, 116.4, 61.8 (CHCH$_2$), 42.9 (CHCH$_2$). Anal. $C_{25}H_{18}BrN_3O_2S$ (C, H, N).

*4.2.4.4. 5-(4-Hydroxy-3,5-dimethoxybenzylidene)-2-[5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**16**).* Yield 59% (method A), mp 297–298 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4): δ 9.32 (br s, 2H), 7.88 (d, J = 7.6 Hz, 2H), 7.56 (s, 1H), 7.56–7.42 (m, 3H), 7.12 (t, J = 7.9 Hz, 1H), 6.96 (d, J = 8.0 Hz, 1H), 6.94 (s, 2H), 6.84 (d, J = 8.0 Hz, 1H), 6.75 (t, J = 7.9 Hz, 1H), 5.90 (dd, J = 11.6, 4.1 Hz, 1H), 4.09 (dd, J = 17.9, 11.6 Hz, 1H), 3.38 (dd, J = 17.9, 4.1 Hz, 1H), 3.92 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 180.1 (C=O), 170.5 (C=N, thiaz.), 162.1 (C=N, pyraz.), 155.1 (C–OH), 148.9, 138.8, 132.6, 132.2, 130.5, 129.7, 128.1, 126.2, 125.3, 124.8, 119.7, 116.4, 108.4, 61.5, (CHCH$_2$), 56.9 (O–CH$_3$), 42.9 (CHCH$_2$). EI-MS (m/z): 501 (M^+). Anal. $C_{27}H_{23}N_3O_5S$ (C, H, N).

*4.2.4.5. 5-(4-Nitrobenzylidene)-2-[5-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**17**).* Yield 73% (method A), 69% (method B), mp 293–294 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4): δ 8.35 (d, J = 8.7 Hz, 2H), 7.96 (d, J = 8.7 Hz, 4H), 7.75 (s, 1H), 7.59–7.52 (m, 3H), 7.22 (d, J = 8.6 Hz, 2H), 6.92 (d, J = 8.6 Hz, 2H), 5.88 (dd, J = 11.1, 3.7 Hz, 1H), 4.17 (dd, J = 18.3, 11.1 Hz, 1H), 3.71 (s, 3H), 3.40 (dd, J = 18.3, 3.7 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 179.3 (C=O), 170.5 (C=N, thiaz.), 162.9 (C=N, pyraz.), 159.7 (C–OH), 147.8, 140.9, 132.9, 132.6, 132.5, 131.3, 130.1, 129.7, 128.9, 128.2, 128.1, 124.9, 114.9, 64.3 (CHCH$_2$), 55.8 (O–CH$_3$), 44.2 (CHCH$_2$). EI-MS (m/z): 484 (M^+). Anal. $C_{26}H_{20}N_4O_4S$ (C, H, N).

*4.2.4.6. 5-(4-Dimethylaminobenzylidene)-2-[5-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]-1,3-thiazol-4(5*H*)-one (**18**).* Yield 59% (method A), 65% (method B), mp 258–259 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4): δ 7.88 (d, J = 7.8 Hz, 2H), 7.56 (s, 1H), 7.55–7.42 (m, 5H), 7.19 (d, J = 8.6 Hz, 2H), 6.91 (d, J = 8.6 Hz, 2H), 6.82 (d, J = 8.8 Hz, 2H), 5.81 (dd, J = 11.2, 4.0 Hz, 1H), 4.12 (dd, J = 18.4, 11.2 Hz, 1H), 3.71 (s, 3H), 3.45 (dd, J = 18.4, 4.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 187.5 (C=O), 177.9 (C=N, thiaz.), 161.2 (C=N, pyraz.), 159.5 (C–OH), 152.1 (4-NMe$_2$), 133.1, 132.7, 132.2, 130.5, 129.7, 127.9, 127.8, 114.9, 112.7, 63.9 (CHCH$_2$), 55.8 (O–CH$_3$), 44.0 (CHCH$_2$). EI-MS (m/z): 482 (M^+). Anal. $C_{28}H_{26}N_4O_2S$ (C, H, N).

4.3. Pharmacology

Primary anticancer assay was performed at approximately 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 [18–22]. 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. 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% (GI_{50}) 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 more 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 logs 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 **5**

Crystal data: $C_{28}H_{25}BrN_4O_3S$, $M_r = 577.49$, triclinic, space group *P*-1, $a = 8.5393(12)$, $b = 12.3835(19)$, $c = 12.8118(15)$ Å, $\alpha = 96.085(11)$, $\beta = 94.681(11)$, $\gamma = 91.218(12)^\circ$, $V = 1342.1(3)$ Å^3, $T = 293(2)$ K, $Z = 2$.

Data collection. A yellow block crystal of $0.50 \times 0.27 \times 0.24$ mm was used to record 5080 (Cu Kα radiation, $\theta_{max} = 70.1^\circ$) intensities on a Kuma KM-4 diffractometer [28]. Accurate unit cell parameters were determined by the least squares refinement fit to the setting angles of 55 reflections collected in the range $15.4 \leq \theta \leq 29.1^\circ$. The intensities were collected in the ω–2θ scan mode with graphite-monochromatized Cu Kα radiation. The intensities were corrected for Lorentz, polarization effects, and absorption using an empirical model derived from psi scans [29] (μ(Cu Kα) = 3.116 mm^{-1}). The minimum and maximum transmissions were 0.188 and 0.473. The 4857 total unique reflections (R(int) = 0.028) were used for further calculations. The position of the H atom bonded to O atom in the hydroxyphenyl moiety was obtained from difference Fourier map and was refined freely. The remaining H atoms were positioned geometrically, and were refined with a riding model (C–H = 0.93–0.96 Å 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 group, which was allowed to rotate.

Structure solution and refinement. The structure was solved by the direct methods using the program SHELXS-97 [30], and refinement was done against F^2 for all data using SHELXL-97 [30]. The final refinement converged with $R = 0.0468$ (for 4013 data with $F^2 > 4\sigma(F^2)$), $wR = 0.1463$ (on F^2 for all data), and $S = 1.079$ (on F^2 for all data). The largest difference peak and hole were 0.782 and −0.834 e Å^{-3}. The molecular illustration was drawn using ORTEP-3 for Windows [31]. Software used to prepare material for publication was WINGX [32].

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 676662).

Acknowledgements

We are grateful to Dr. V.L. Narayanan from Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, MD, USA, for *in vitro* evaluation of anticancer activity. This work has been partially supported by the Polish National Commission for UNESCO (fellowship for D. Havrylyuk) and by Jozef Mianowski Fund – Foundation for the Promotion of Science (fellowship for R. Lesyk).

References

[1] R.B. Lesyk, B.S. Zimenkovsky, Curr. Org. Chem. 8 (2004) 1547–1577.
[2] G.I. Murphy, J.C. Holder, Trends Pharmacol. Sci. 21 (2000) 469–474.
[3] C. Charlier, C. Mishaux, Eur. J. Med. Chem. 38 (2003) 645–659.
[4] Kaoru Seno, Takayuki Okuno, Koichi Nishi, Yasushi Murakami, Fumihiko Watanabe, Takaharu Matsuura, Masaaki Wada, Yasuhiko Fujii, Masaaki Yamada, Tomoyuki Ogawa, Tetsuo Okada, Hiroshi Hashizume, Makoto Kii, Shin-ichiro Hara, Sanji Hagishita, Shozo Nakamoto, Katsutoshi Yamada, Yukiko Chikazawa, Masahiko Ueno, Isao Teshirogi, Takashi Ono, Mitsuaki Ohtani, J. Med. Chem. 43 (2000) 1041–1044.
[5] Mui Mui Sim, Siew Bee Ng, A.D. Buss, S.C. Crasta, Kah Lin Goh, Sue Kim Lee, Bioorg. Med. Chem. Lett. 12 (2002) 679–699.
[6] N.S. Cutshall, C. O'Day, M. Prerzhdo, Bioorg. Med. Chem. Lett. 15 (2005) 3374–3379.
[7] 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, Proc. Natl. Acad. Sci. U.S.A. 98 (21) (2001) 11879–11884.
[8] A. Degterev, A. Lugovskoy, M. Cardone, B. Mulley, G. Wagner, T. Mitchison, J. Yuan, Nat. Cell Biol. 3 (2) (2001) 173–182.
[9] R. Dayam, F. Aiello, J. Deng, Y. Wu, A. Garofalo, X. Chen, N. Neamati, J. Med. Chem. 49 (2006) 4526–4534.
[10] R. Lesyk, B. Zimenkovsky, D. Atamanyuk, F. Jensen, K. Kiec-Kononowicz, A. Gzella, Bioorg. Med. Chem. 14 (2006) 5230–5240.
[11] R. Lesyk, O. Vladzimirska, S. Holota, L. Zaprutko, A. Gzella, Eur. J. Med. Chem. 42 (2007) 641–648.
[12] D. Atamanyuk, B. Zimenkovsky, R. Lesyk, J. Sulfur Chem. 29 (2008) 151–162.
[13] R. Lesyk, B. Zimenkovsky, I. Subtelna, I. Nektegayev, G. Kazmirchuk, Acta Polon. Pharm. – Drug Res. 6 (2003) 457–466.
[14] A. Özdemir, G. Turan-Zitouni, Z.A. Kaplancikli, G. Revial, K. Güven, Eur. J. Med. Chem. 42 (2007) 403–409.
[15] K. Popov-Pergal, Z. Cekovic, M. Pergal, J. Gen. Chem. USSR 61 (1991) 1958–1962.
[16] F.H. Allen, O. Kennard, D.G. Watson, L. Brammer, A.G. Orpen, R. Taylor, J. Chem. Soc., Perkin Trans. 2 (1987) S.1–19.
[17] D. Cremer, J.A. Pople, J. Am. Chem. Soc. 97 (1975) 1354–1358.
[18] <http://dtp.nci.nih.gov>.
[19] A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, J. Nat. Cancer Inst. 83 (11) (1991) 757–766.
[20] M.R. Boyd, K.D. Paull, Drug Dev. Res. 34 (1995) 91–109.
[21] M.R. Boyd, in: B.A. Teicher (Ed.), Cancer Drug Discovery and Development, vol. 2, Humana Press, 1997, pp. 23–43.
[22] R.H. Shoemaker, Nat. Rev. Cancer 6 (2006) 813–823.
[23] R. Laske, H. Schönenberger, E. Holler, Arch. Pharm. 322 (12) (1989) 857–862.
[24] A.J. Kemp, S.D. Lyons, R.I. Christopherson, J. Biol. Chem. 261 (32) (1986) 14891–14895.
[25] Org. Synth. 3 (1955) 763.
[26] (a) I.D. Komaritsa, S.N. Baranov, A.P. Grishchuk, Khimiya Geterotsiklicheskikh Soedinenii 4 (1967) 664–665;
(b) Chem. Abstr. 68 (1968) 49495.
[27] E. Palaska, V. Aytemir, I.T. Uzbay, D. Erol, Eur. J. Med. Chem. 36 (2001) 539–543.
[28] Kuma Diffraction, Version 8.0.1, KM-4 Software User's Guide, Wrocław, Poland, 1996.
[29] A.C.T. North, D.C. Phillips, F.S. Mathews, Acta Crystallogr. A24 (1968) 351 – 359.
[30] G.M. Sheldrick, SHELXS-97 and SHELXL-97. Release 97-2, University of Göttingen, Germany, 1997.
[31] L.J. Farrugia, J. Appl. Crystallogr. 30 (1997) 565.
[32] L.J. Farrugia, J. Appl. Crystallogr. 32 (1999) 837–838.