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Original article

New 5-substituted thiazolo[3,2-*b*][1,2,4]triazol-6-ones: Synthesis and anticancer evaluation

Roman Lesyk [a,*], Olena Vladzimirska [a], Serhiy Holota [a], Lucjusz Zaprutko [b], Andrzej Gzella [b]

[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*

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Abstract

Following [2+3]-cyclocondensation reaction of 1,2,4-triazole-3(5)-thiol with *N*-arylmaleimides or with monochloroacetic acid and oxocompounds, *N*-(*R*-phenyl)-(6-oxo-5,6-dihydro[1,3]thiazol[3,2-*b*][1,2,4]triazol-5-yl)acetamides (**1**–**5**) and 5-ylidene-[1,3]thiazolo[3,2-*b*][1,2,4]triazol-6-ones (**6**–**11**) were synthesized as possible anticancer agents. Anticancer activity evaluation on the full panel of nearly 60 human cancer cell lines showed that synthesized compounds displayed this kind of activity on renal cancer, leukemia, colon cancer, breast cancer and melanoma cell lines. It was shown that 5-ylidene-[1,3]thiazolo[3,2-*b*][1,2,4]triazol-6-ones are characterized with more potent anticancer activity than respective amides. The structures of the compounds were determined by ^{1}H NMR, ^{13}C NMR and X-ray analysis.

Keywords: Thiazolo[3,2-*b*][1,2,4]triazol-6-ones; X-ray study; Anticancer activity

1. Introduction

Design and synthesis of novel small molecules which can specifically block some targets in tumor cells are in perspective direction in modern medicinal chemistry. Many synthetic small molecules from different groups of heterocycles with influence on carcinogenesis have been reported and several of them are currently in clinical trials [1,2]. 1,2,4-Triazole and 4-thiazolidone moieties are perspective scaffolds for design of anticancer drugs (Fig. 1). Thus, 3-arylamine-5-(hetero)aryl-1,2,4-triazoles are known as novel class of potent tubulin polymerization inhibitors that bind to the colchicine site on tubulin [3]. Also, 3-*S*-alkylated-5-(hetero)aryl-1,2,4-triazoles are agonists of somatostatin sst2/sst5 receptors, which mediate antiproliferative activity of somatropin release-inhibiting factor [4,5]. In turn, inhibitors of antiapoptotic proteins Bcl-X_L and BH3 which regulate apoptosis have been identified among some 5-arylidene derivatives of 3-alkancarboxylic-2-thioxo-4-thiazolidones [6–8]. 3-Alkyl-5-heteroarylidene-4-thiazolidones inhibit binding of tumor necrosis factor alpha (TNFα) to the type-1 TNF receptor (TNFRc-1) [9]. The anticancer effects of some 5-benzylidene-thiazolidine-2,4-diones and -thiones are mediated by partial depletion of intracellular Ca^{2+} stores leading to inhibition of translation initiation, phosphorylation of eIF2α leads to preferential down regulation of G1 cyclins and other oncogenic proteins, and to cell cycle arrest in G1 phase [10]. For a search of new effective small molecules with anticancer effect it seems to be interesting to combine above-mentioned heterocyclic scaffolds into the one system – thiazolo[3,2-*b*][1,2,4]triazol-6-one and to evaluate anticancer activity of synthesized compounds.

2. Chemistry

Aiming the synthesis of target heterocycles we used an approach to obtaining thiazolo[3,2-*b*][1,2,4]triazol-6-one system, which relies on [2+3]-cyclocondensation of 1,2,4-triazole-3(5)-thiol with equivalents of dielectrophilic synthon $[C_2]^{2+}$.

* Corresponding author. Tel.: +38 0322 75 77 34; fax: +38 0322 74 77 34.
E-mail address: dr_r_lesyk@org.lviv.net (R. Lesyk).

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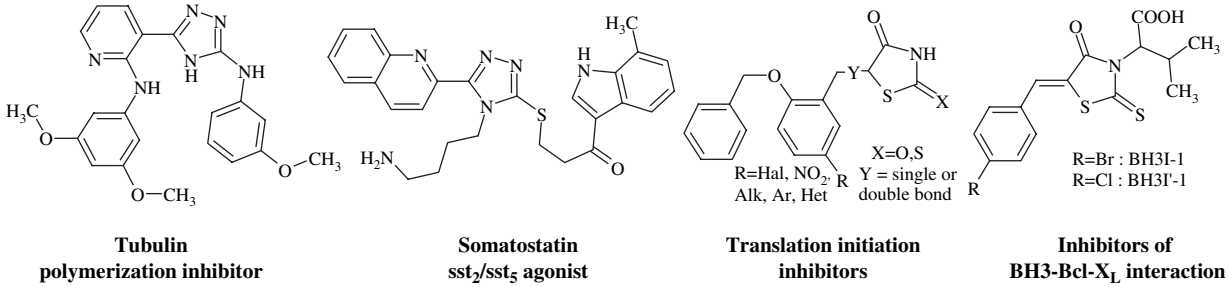


Fig. 1. 1,2,4-Triazoles and 4-thiazolidones as potential anticancer agents.

We first studied interaction of above-mentioned binucleophile with *N*-arylmaleimides and also used well-known reaction with monochloroacetic acid and oxocompounds (isatin, some *p*-substituted benzaldehydes, cinnamaldehyde). The oxocompounds' selection has been reasoned by their clearly defined influence on realization of antitumor effect for 4-thiazolidones in accordance with our previous SAR studies [11]. As result, group of new 5-substituted thiazolo[3,2-*b*][1,2,4]triazol-6-ones have been synthesized (Scheme 1).

3. Results and discussion

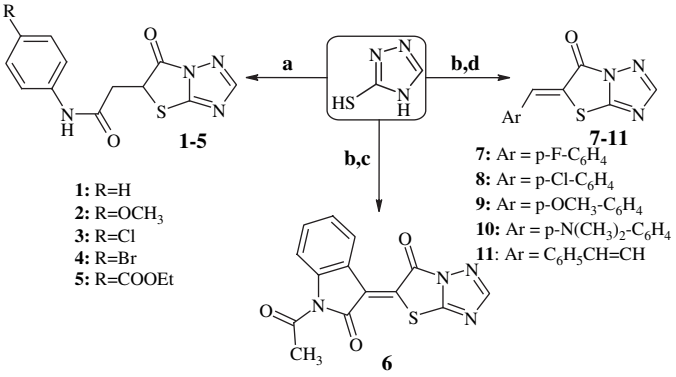
The characterization data of synthesized novel 5-substituted thiazolo[3,2-*b*][1,2,4]triazol-6-ones are given in experimental part. Analytical and spectral data (^{1}H NMR, ^{13}C NMR) confirmed the structure of the synthesized compounds.

In the ^{1}H NMR spectra of compounds **1**–**5** protons of CH_2–CH fragment show characteristic patterns of an ABX system. The chemical shifts of the protons H_A, H_B, and H_X have been assigned to about $\delta \sim 3.20$–3.40, $\delta \sim 2.90$–3.00, and $\delta \sim 4.50$–4.70, respectively, and indicate that the methylene protons adjacent to an asymmetric centre are magnetically non-equivalent. The large values of J_{AB}'s (approximate coupling constants of $J_{AB} = 17.0$–18.0, $J_{AX} = 5.0$–7.5, and $J_{BX} = 4.0$–5.0 Hz) were here observed like as in the structurally related 2-thioxo-4-thiazolidones which were early referred to as "carbonyl effect" on the coupling constants for the methylene groups by Takahashi [12]. The ^{1}H NMR spectra of **1**–**11** showed a singlet at $\delta \sim 8.30$–8.40 corresponding to the proton of triazole nucleus. The chemical shift for the methylidene group of compounds **7**–**10** is insignificantly displaced in weak magnetic field, $\delta \sim 8.20$, and clearly indicated that only *Z*-isomers were obtained [13].

Regioselectivity of [2+3]-cyclocondensation of 1,2,4-triazole-3(5)-thiol with above-mentioned equivalents of dielectrophilic synthon $[C_2]^{2+}$ was confirmed by X-ray crystallographic analysis of exemplified compound **10**. Its molecular structure and the atom-labelling scheme are presented in Fig. 2.

The eight-membered thiazolo-triazole system is planar with an r.m.s. deviation of 0.0064 Å. The *N*,*N*-dimethylaminophenyl moiety is in the *cis* (*Z*) configuration with respect to the S atom of the fused thiazolo[3,2-*b*][1,2,4]triazol-6-one system. In the *N*,*N*-dimethylaminophenyl moiety the dimethylamino group is essentially coplanar with the six-membered ring; the interplanar angle between N16/C17/C18 and C10–C15 planes amounts to 1.9(3)°. Simultaneously, the phenyl ring is twisted slightly out of the plane of the fused thiazolo-triazole system. The dihedral angle between these two named systems is 7.27(9)°.

Due to the electron-donating effect of dimethylamino group the phenyl ring exhibit noticeable quinoid character that is demonstrated by the shortening of the C11–C12 [1.374(2) Å] and C14–C15 [1.368(2) Å] bond lengths compared to the standard C_{ar}–C_{ar} distance of 1.397(1) Å [14]. In addition, the exocyclic C9–C10 [1.433(2) Å] and C13–N16 [1.359(2) Å] bonds are shortened with respect to the normal lengths of the (C=)C–C_{ar} [1.470(2) Å] and C(sp^2)–N(–C_2) [1.371(2) Å] by about 13 and 4σ, respectively.



Scheme 1. Synthesis of 5-substituted thiazolo[3,2-*b*][1,2,4]triazole-6-ones. Reagents: (a) *N*-arylmaleimides (1.0 eq.), AcOH; (b) $ClCH_2COOH$ (1.0 eq.), AcONa (2.0 eq.), AcOH:Ac_2O (1:1); (c) isatin; (d) Ar–CHO.

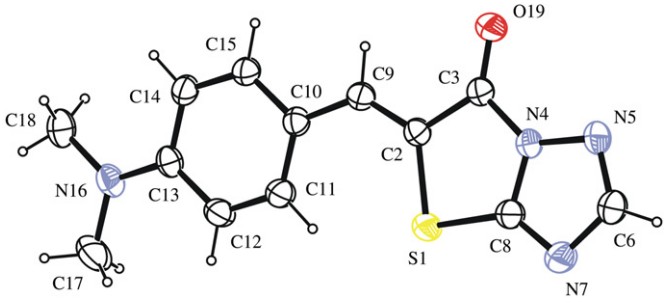


Fig. 2. X-ray structure of the compound **10**.

4. Anticancer evaluation

Anticancer assays of the compounds **1**–**5**, **7**, **8**, **11** were performed according to the US NCI protocol, as described elsewhere [15–17]. These substances were evaluated in the 3-cell line panel consisting of NCI-H460 (lung), MCF7 (breast), and SF-268 (CNS) cell lines. As a result seven synthesized substances successfully passed pre-screening phase. Only compound **11** was found to be inactive in the pre-screening conditions. It is interesting that almost all active substances showed dominant growth inhibition activity against lung and breast cancer cell lines.

Compounds **1**–**5**, **7**, **8** were consequently selected for in vitro testing against the full panel of nearly 60 cell lines. The synthesized *N*-(*R*-phenyl)-(6-oxo-5,6-dihydro[1,3]-thiazol[3,2-*b*][1,2,4]triazol-5-yl)acetamides (**1**–**5**) displayed moderate activity in the in vitro screen on renal cancer, leukemia, colon cancer, breast cancer and melanoma cell lines but low activity on CNS cancer, ovarian cancer and prostate cancer cell lines. It is noteworthy that there is observed selective influence of compounds on some cancer cell lines, depending on the nature of the substituent R in the phenylacetamide fragment. The unsubstituted derivative **1** was highly active on a non-small cell lung cancer (NCI-H23, $\log GI_{50}$ value −6.94), colon cancer (SW-620, $\log GI_{50}$ value −5.49) and breast cancer (BT-549, $\log GI_{50}$ value −6.47). And also compounds **4** (R = Br) and **5** (R = COOEt) were highly active on the breast cancer (HS 578T, $\log GI_{50}$ value −5.14) and leukemia (RPMI-8226, $\log GI_{50}$ value −5.34) cells, respectively. 5-Ylidene-[1,3]thiazolo[3,2-*b*][1,2,4]triazol-6-ones have shown more potent anticancer activity than amides **1**–**5**. So, compound **8** (Ar = *p*-Cl-C_6H_4) was highly active on all cell lines, especially on leukemia (for all lines $\log GI_{50}$ value less than −5.00), and exchange of the chlorine atom in 5-arylidene moiety for fluorine (**7**) gives decrease of activity against most of cancer cell lines, besides leukemia (HL-60 (TB), $\log GI_{50}$ value −5.19).

The results of the primary screening and the full panel screening of some synthesized compounds are summarized in Tables 1 and 2.

5. Conclusion

In the present paper, 11 new 5-substituted thiazolo [3,2-*b*][1,2,4]triazol-6-ones were described. Reaction of 1,2, 4-triazole-3(5)-thiol with *N*-arylmaleimides yielding *N*-(*R*-phenyl)-(6-oxo-5,6-dihydro[1,3]thiazol[3,2-*b*][1,2,4]triazol-5-yl)acetamides (**1**–**5**) was proposed as approach to new thiazo lo[3,2-*b*][1,2,4]triazol-6-ones. Seven of synthesized thiazolo [3,2-*b*][1,2,4]triazol-6-ones (**1**–**5**, **7**, **8**) were tested and most of them displayed antitumor activity on renal cancer, leukemia, colon cancer, breast cancer and melanoma cell lines. 5-(4-Chlorobenzylidene)-[1,3]thiazolo[3,2-*b*][1,2,4]triazol-6-one (**8**) with selective influence on leukemia cell lines was identified. The results of this study prompt us to in-depth anticancer studies of these bicyclic condensed heterocycles.

6. Experimental

6.1. Materials and methods

The starting 1,2,4-triazole-3(5)-thiol [18] and *N*-arylmaleimides [19] 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, and N) were performed using the Perkin–Elmer 2400 CHN analyzer and were within ±0.4% of the theoretical values. The ^1H and ^{13}C NMR spectra were recorded on Varian Gemini spectrometer at 300 MHz and 75 MHz, respectively, with a mixture of DMSO-d_6 + CCl_4 as a solvent and TMS as an internal standard. Chemical shifts values are reported in parts per million units with use of δ scale.

6.2. Chemistry

6.2.1. General procedure for synthesis of N-(R-phenyl)-(6-oxo-5,6-dihydro[1,3]thiazolo[3,2-b][1,2,4]triazol-5-yl)-acetamides (1–5)

Mixtures of 1,2,4-triazole-3(5)-thiol (10 mmol) and appropriate *N*-arylmaleimides (10 mmol) were refluxed for 2 h in

Table 1
In vitro anticancer activity for compounds **1**–**5**, **7**, **8**, **11**

Compound	3-Cell lines assay[a]			N^b	$\log GI_{50}$			log TGI			$\log LC_{50}$		
	A	*B*	*C*		$N1^b$	Range	MG_MID	$N2^b$	Range	MG_MID	$N3^b$	Range	MG_MID
1	0	110	0	49	36	−6.94 to −4.14	−4.51	25	−6.04 to −4.07	−4.21	10	−5.23 to −4.01	−4.05
2	0	107	0	56	46	−4.78 to −4.01	−4.48	34	−4.51 to −4.10	−4.21	19	−4.26 to −4.02	−4.05
3	0	107	0	56	35	−4.83 to −4.04	−4.38	29	−4.51 to −4.02	−4.14	7	−4.25 to −4.03	−4.02
4	0	100	0	57	47	−5.14 to −4.18	−4.56	36	−4.55 to −4.01	−4.23	20	−4.28 to −4.03	−4.07
5	0	8	0	45	33	−5.34 to −4.02	−4.42	23	−5.01 to −4.06	−4.18	10	−4.27 to −4.03	−4.04
7	13	70	77	57	51	−5.19 to −4.04	−4.29	2	−4.10, −4.03	−4.00	–	–	−4.00
8	3	11	10	57	57	−6.54 to −4.53	−4.94	56	−5.69 to −4.06	−4.50	39	−4.31 to −4.01	−4.12
11	95	128	138						Inactive				

[a] The results for each compound are reported as the growth percent of treated cells when compared to untreated control cells accordingly for the following human tumor cell lines: (A) NCI-H460 (lung cancer); (B) SF-268 (CNS cancer) and (C) MCF7 (breast cancer).

[b] N – Number of human tumor cell lines tested at the second stage; *N*1, *N*2, *N*3 – number of cell lines sensitive to this compound ($\log GI_{50} < -4.00$).

Table 2
In vitro anticancer activity in 60 human tumor cell lines for compounds **1**–**5**, **7**, **8**

Panel/cell line	log GI_{50}/log TGI						
	1	**2**	**3**	**4**	**5**	**7**	**8**
Leukemia							
CCRF-CEM	−4.47/>−4.00	−4.69/−4.38	−4.72/−4.38	−4.67/−4.26	−4.61/−4.11	–	−6.45/−4.70
HL-60 (TB)	–	−4.56/>−4.00	–	−4.71/>−4.00	−4.33/>−4.00	−5.19/>−4.00	−6.27/−5.69
K-562	–	−4.50/>−4.00	−4.72/−4.36	−4.58/−4.10	−4.02/>−4.00	−4.41/>−4.00	−5.12/−4.54
MOLT-4	–	−4.54/−4.15	−4.70/−4.32	−4.60/−4.01	−4.47/−4.18	−4.41/>−4.00	−5.79/−5.06
RPMI-8226	−4.68/−4.19	−4.60/−4.19	−4.62/−4.00	−4.63/−4.19	−5.34/−5.01	−4.58/−4.10	−5.53/−4.76
SR	–	–	–	−4.74/>−4.00	–	−4.81/>−4.00	−5.08/−4.47
Non-small cell lung cancer							
A549/ATCC	>−4.00/>−4.00	−4.21/>−4.00	>−4.00/>−4.00	−4.45/>−4.00	>−4.00/>−4.00	−4.06/>−4.00	−4.67/−4.08
EKVX	−/−4.19	−4.45/>−4.00	>−4.00/>−4.00	−4.50/>−4.00	−4.55/−4.16	−4.08/>−4.00	−4.84/−4.47
HOP-62	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	−4.67/−4.32	−4.15/>−4.00	−4.80/−4.45
HOP-92	−4.60/−4.18	−4.66/−4.31	−4.60/−4.19	−4.65/−4.31	−4.71/−4.32	−4.32/>−4.00	–
NCI-H226	–	−4.64/−4.37	−4.04/>−4.00	−4.70/−4.34	−4.58/−4.18	>−4.00/>−4.00	−4.70/−4.26
NCI-H23	−6.94/−5.04	−4.70/−4.43	−4.63/−4.15	−4.68/−4.36	–	–	−4.75/−4.42
NCI-H322M	>−4.00/>−4.00	−4.01/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	−4.88/−4.58
NCI-H460	−4.45/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	−4.21/>−4.00	−4.76/−4.35
NCI-H522	−4.66/−4.37	−4.71/−4.36	−4.57/−4.19	−4.69/−4.24	–	−4.30/>−4.00	−5.27/−4.73
Colon cancer							
COLO 205	−4.59/−4.10	−4.52/−4.10	−4.71/−4.32	−4.75/−4.43	−4.67/−4.39	−4.21/>−4.00	−4.77/−4.41
HCC-2998	–	>−4.00/>−4.00	−4.42/>−4.00	>−4.00/>−4.00	−4.74/−4.49	−4.20/>−4.00	−4.74/−4.49
HCT-116	−4.49/>−4.00	−4.77/−4.51	−4.70/−4.51	−4.80/−4.53	−4.80/−4.53	−4.47/>−4.00	−4.97/−4.59
HCT-15	−4.48/>−4.00	−4.61/−4.28	−4.80/−4.39	−4.83/−4.55	−4.68/−4.28	−4.20/>−4.00	−4.92/−4.46
HT29	>−4.00/>−4.00	−4.32/>−4.00	−4.54/−4.12	−4.55/>−4.00	−4.36/>−4.00	−4.34/>−4.00	−4.82/−4.23
KM12	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	−4.45/>−4.00	−4.98/−4.59
SW-620	−5.49/−4.35	−4.43/>−4.00	−4.49/−4.10	−4.35/>−4.00	−4.41/>−4.00	−4.18/>−4.00	−4.83/−4.51
CNS cancer							
SF-268	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	−4.47/>−4.00	−4.79/−4.23
SF-295	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	−4.28/>−4.00	−4.75/−4.45
SF-539	–	−4.57/−4.26	−4.50/−4.13	−4.68/−4.33	–	−4.52/−4.03	−4.99/−4.66
SNB-19	>−4.00/>−4.00	–	>−4.00/>−4.00	–	>−4.00/>−4.00	−4.20/>−4.00	−4.79/−4.49
SNB-75	−4.50/−4.07	–	–	–	–	−4.23/>−4.00	−4.85/−4.49
U251	>−4.00/>−4.00	−4.65/−4.35	−4.74/−4.47	−4.78/−4.50	>−4.00/>−4.00	−4.22/>−4.00	−4.98/−4.63
Melanoma							
LOX IMVI	−4.59/−4.27	−4.66/−4.37	−4.69/−4.34	−4.79/−4.50	−4.74/−4.45	−4.43/>−4.00	−4.97/−4.60
MALME-3M	−4.72/−4.35	−4.76/−4.47	−4.83/−4.41	−4.84/−4.50	–	>−4.00/>−4.00	−4.95/−4.57
M14	−4.66/−4.34	−4.74/−4.47	−4.45/>−4.00	−4.80/−4.53	−4.73/−4.46	−4.54/>−4.00	−4.82/−4.45
SK-MEL-2	−4.66/−4.27	>−4.00/>−4.00	>−4.00/>−4.00	−4.18/>−4.00	–	−4.29/>−4.00	−4.90/−4.55
SK-MEL-28	>−4.00/>−4.00	−4.57/−4.24	−4.64/−4.24	−4.63/−4.15	−4.04/>−4.00	−4.28/>−4.00	−4.78/−4.45
SK-MEL-5	−4.64/−4.32	−4.76/−4.45	>−4.00/>−4.00	−4.73/−4.41	>−4.00/>−4.00	−4.38/>−4.00	−4.97/−4.63
UACC-257	−4.39/>−4.00	−4.70/−4.44	−4.39/>−4.00	−4.76/−4.48	−4.06/>−4.00	−4.02/>−4.00	−4.87/−4.52
UACC-62	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	−4.39/>−4.00	−4.18/>−4.00	−4.84/−4.53
Ovarian cancer							
IGROV1	−4.59/−4.18	−4.17/>−4.00	>−4.00/>−4.00	−4.46/>−4.00	–	−4.24/>−4.00	−5.00/−4.61
OVCAR-3	−4.46/>−4.00	−4.73/−4.42	−4.66/−4.29	−4.70/−4.43	−4.34/>−4.00	−4.43/>−4.00	−4.88/−4.52

OVCAR-4	>−4.00/>−4.00	−4.24/>−4.00	−4.50/−4.08	−4.50/>−4.00	−	−4.46/>−4.00	−4.76/−4.20
OVCAR-5	−	−4.74/−4.49	−4.65/−4.25	−4.79/−4.53	−4.77/−4.50	−4.26/>−4.00	−4.70/−4.46
OVCAR-8	−4.59/−4.22	−4.65/−4.33	>−4.00/>−4.00	−4.74/−4.42	>−4.00/>−4.00	−4.35/>−4.00	−4.90/−4.44
SK-OV-3	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	>−4.00/>−4.00	−	>−4.00/>−4.00	−4.53/−4.26
Renal cancer							
786-0	−4.71/−4.44	−4.78/−4.51	−4.69/−4.36	−4.78/−4.52	−4.81/−4.54	−4.29/>−4.00	−4.87/−4.54
A498	−	−4.67/−4.24	>−4.00/>−4.00	−4.91/−4.24	−	−4.21/>−4.00	−4.72/−4.48
ACHN	−4.66/−4.38	−4.67/−4.32	−4.76/−4.51	−4.82/−4.55	−4.76/−4.50	−4.09/>−4.00	−4.84/−4.41
CAKI-1	−4.63/−4.31	−4.73/−4.46	>−4.00/>−4.00	−4.69/−4.36	−4.20/>−4.00	−4.16/>−4.00	−4.71/−4.41
RXF 393	−4.73/−4.37	−4.81/−4.38	−4.60/−4.29	−4.89/−4.50	−4.67/−4.17	−4.33/>−4.00	−5.28/−4.74
TK-10	−4.72/−4.40	−4.78/−4.51	−4.68/−4.40	−4.76/−4.50	−4.64/−4.26	>−4.00/>−4.00	−4.78/−4.50
UO-31	−4.60/−4.24	−4.63/−4.27	−4.64/−4.34	−4.73/−4.42	−	−4.16/>−4.00	−4.91/−4.48
SN12C	−4.56/−4.17	−4.60/−4.25	−4.64/−4.27	−4.64/−4.20	−4.58/−4.06	−4.39/>−4.00	−4.81/−4.31
Prostate cancer							
PC-3	−4.14/>4.00	−4.63/−4.25	−4.26/>4.00	−4.57/−4.02	−4.74/−4.39	−4.27/>−4.00	−4.93/−4.54
DU-145	−	−4.24/>4.00	>−4.00/>−4.00	−4.41/>4.00	−	−4.40/>−4.00	−4.89/−4.49
Breast cancer							
MCF7	−4.44/>4.00	−4.60/−4.19	−4.36/>4.00	−4.57/−4.13	−4.41/>4.00	−4.49/>−4.00	−5.20/−4.51
NCI/ADR-RES	−4.20/>4.00	−4.50/>4.00	−4.42/>4.00	−4.60/−4.15	−4.74/−4.44	−4.42/>−4.00	−4.58/−4.05
HS 578T	−4.91/−4.34	−4.59/−4.35	>−4.00/>−4.00	−5.14/−4.70	>−4.00/>−4.00	−4.16/>−4.00	−4.85/−4.43
MDA-MB-435	−4.38/>4.00	−4.58/−4.15	−4.26/>4.00	−4.50/>4.00	>−4.00/>−4.00	−4.43/>−4.00	−4.90/−4.57
BT-549	−6.47/−6.00	>−4.00/>−4.00	−4.43/−4.02	>−4.00/>−4.00	−4.66/−4.36	−4.37/>−4.00	−
T-47D	−4.40/>4.00	−4.43/>4.00	−4.62/−4.21	−4.74/−4.34	−4.63/−4.24	−4.04/>−4.00	−4.67/>4.00
MDA-MB-231/ATCC	−4.72/−4.41	−4.67/−4.41	−4.50/−4.08	−4.77/−4.44	−	>−4.00/>−4.00	−4.72/−4.36

10 ml of glacial acetic acid. After cooling to the room temperature, reaction mixtures were poured into 50 ml of water. Precipitated white powders were filtered off, washed with methanol and recrystallized with ethanol (5–15 ml).

*6.2.1.1. 2-(6-Oxo-5,6-dihydro[1,3]thiazolo[3,2-b][1,2,4]triazol-5-yl)-N-phenylacetamide (**1**).* Yield 57%, mp 163–165 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4, δ): 14.10 (s, 1H), 8.40 (s, 1H), 7.50 (t, $J = 8.0$ Hz, 2H), 7.40 (t, $J = 8.0$ Hz, 1H), 7.30 (d, $J = 8.0$ Hz, 2H), 4.50 (m, 1H), 3.40 (dd, $J = 17.0$, 5.0 Hz, 1H), 2.90 (dd, $J = 17.0$, 4.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 174.6 (C=O), 174.2 (C=O), 156.5, 145.5, 132.7, 128.8, 128.3, 126.9, 41.1, 36.5. Calc. for $C_{12}H_{10}N_4O_2S$, %: C, 52.55; H, 3.67; N, 20.43. Found, %: C, 52.49; H, 3.61; N, 20.37.

*6.2.1.2. N-(4-Methoxyphenyl)-(6-oxo-5,6-dihydro[1,3]thiazolo-[3,2-b][1,2,4]triazol-5-yl)acetamide (**2**).* Yield 46%, mp 158–159 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4, δ): 14.32 (s, 1H), 8.27 (s, 1H), 7.14 (d, $J = 8.1$ Hz, 2H), 7.05 (d, $J = 8.1$ Hz, 2H), 4.67 (m, 1H), 3.80 (s, 3H), 3.40 (dd, $J = 17.2$, 6.2 Hz, 1H), 2.92 (dd, $J = 17.2$, 4.3 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 175.2 (C=O), 174.4 (C=O), 170.2, 165.5, 158.9, 145.4, 140.4, 128.1, 125.3, 123.9, 55.4, 41.2, 36.4. Calc. for $C_{13}H_{12}N_4O_3S$, %: C, 51.31; H, 3.97; N, 18.41. Found, %: C, 51.26; H, 3.87; N, 18.37.

*6.2.1.3. N-(4-Chlorophenyl)-(6-oxo-5,6-dihydro[1,3]thiazolo-[3,2-b][1,2,4]triazol-5-yl)acetamide (**3**).* Yield 71%, mp 180–182 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4, δ): 14.20 (s, 1H), 8.40 (s, 1H), 7.50 (d, $J = 8.6$ Hz, 2H), 7.30 (d, $J = 8.6$ Hz, 2H), 4.50 (m, 1H), 3.40 (dd, $J = 17.9$, 6.6 Hz, 1H), 2.90 (dd, $J = 17.9$, 4.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 174.0 (C=O), 173.50, (C=O), 169.0, 165.0, 144.5, 139.8, 128.4, 127.6, 40.6, 35.9. Calc. for $C_{12}H_9ClN_4O_2S$, %: C, 46.68; H, 2.94; N, 18.15. Found, %: C, 46.61; H, 2.87; N, 18.07.

*6.2.1.4. N-(4-Bromophenyl)-(6-oxo-5,6-dihydro[1,3]thiazolo-[3,2-b][1,2,4]triazol-5-yl)acetamide (**4**).* Yield 92%, mp 185–187 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4, δ): 14.26 (s, 1H), 8.62 (s, 1H), 7.71 (d, $J = 8.5$ Hz, 2H), 7.25 (d, $J = 8.5$ Hz, 2H), 4.68 (m, 1H), 3.44 (dd, $J = 17.7$, 7.2 Hz, 1H), 2.92 (dd, $J = 17.7$, 4.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 174.5 (C=O), 173.9, (C=O), 169.5, 165.5, 134.7, 131.8, 128.9, 120.4, 41.1, 36.5. Calc. for $C_{12}H_9BrN_4O_2S$, %: C, 40.81; H, 2.57; N, 15.86. Found, %: C, 40.77; H, 2.50; N, 15.79.

*6.2.1.5. Ethyl 4-{[(6-oxo-5,6-dihydro[1,3]thiazolo[3,2-b][1,2,4]-triazol-5-yl)acetyl]amino}benzoate (**5**).* Yield 58%, mp 137–140 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4, δ): 14.20 (s, 1H), 8.40 (s, 1H), 8.10 (d, $J = 8.4$ Hz, 2H), 7.40 (d, $J = 8.4$ Hz, 2H), 4.60 (m, 1H), 4.30 (m, 2H), 3.40 (dd, $J = 18.0$, 7.5 Hz, 1H), 2.90 (dd, $J = 18.0$, 5.0 Hz, 1H), 1.40 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 174.50 (C=O), 173.9, (C=O), 169.4, 165.5, 140.4, 136.7, 129.6, 126.9, 60.8, 36.5, 41.2, 14.6. Calc. for $C_{15}H_{14}N_4O_4S$, %: C, 52.05; H, 4.07; N, 16.18. Found, %: C, 52.11; H, 4.00; N, 16.10.

*6.2.2. General procedure for synthesis of 5-ylidene-[1,3]thiazolo[3,2-b][1,2,4]triazol-6-ones (**6–11**)*

Mixtures of 1,2,4-triazole-3(5)-thiol (10 mmol), monochloroacetic acid (10 mmol), appropriate oxocompounds, namely isatin, *p*-fluoro-, *p*-chloro-, *p*-methoxy-, *p*-dimethylaminobenzaldehydes or cinnamaldehyde (12 mmol) and anhydrous sodium acetate (20 mmol) were refluxed for 3 h in a mixture of acetic anhydride (5 ml) and glacial acetic acid (5 ml). Obtained powders were filtered off, washed with methanol and recrystallized with acetic acid (**7–9**) or DMF:acetic acid 1:3 mixture (**6**, **10**, **11**).

*6.2.2.1. (3Z)-1-Acetyl-3-(6-oxo-[1,3]thiazolo[3,2-b][1,2,4]triazol-5-ylidene)-1,3-dihydro-2H-indol-2-one (**6**).* Yield 40%, red powder, mp 229–230 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4, δ): 8.40 (s, 1H), 9.10 (d, $J = 8.0$ Hz, 1H), 8.30 (d, $J = 8.0$ Hz, 1H), 7.60 (t, $J = 7.6$ Hz, 1H), 7.40 (t, $J = 7.6$ Hz, 1H), 2.60 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 170.86 (C=O), 168.7 (C=O), 162.7, 159.7, 156.6, 135.1, 134.2, 130.0, 127.8, 125.3, 120.5, 115.8, 36.5, 25.1. Calc. for $C_{14}H_8N_4O_3S$, %: C, 53.84; H, 2.58; N, 17.94. Found, %: C, 53.76; H, 2.49; N, 17.87.

*6.2.2.2. (5Z)-5-(4-Fluorobenzylidene)-[1,3]thiazolo[3,2-b][1,2,4]triazol-6-one (**7**).* Yield 53%, yellow powder, mp 198–200 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4, δ): 8.30 (s, 1H), 8.20 (s, 1H), 7.80 (m, 2H), 7.30 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 166.1, 161.1 (C=O), 159.3, 156.1, 138.4, 133.4, 133.2, 128.9, 124.2, 117.1, 116.6. Calc. for $C_{11}H_6FN_3OS$, %: C, 53.44; H, 2.45; N, 16.99. Found, %: C, 53.36; H, 2.39; N, 16.89.

*6.2.2.3. (5Z)-5-(4-Chlorobenzylidene)-[1,3]thiazolo[3,2-b]-[1,2,4]triazol-6-one (**8**).* Yield 68%, yellow powder, mp 177–178 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4, δ): 8.30 (s, 1H), 8.20 (s, 1H), 7.70 (d, $J = 7.8$ Hz, 2H), 7.50 (d, $J = 7.8$ Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 159.4 (C=O), 156.1, 141.4, 138.1, 136.4, 132.3, 131.2, 129.7, 125.4. Calc. for $C_{11}H_6ClN_3OS$, %: C, 50.10; H, 2.29; N, 15.93. Found, %: C, 50.02; H, 2.36; N, 15.86.

*6.2.2.4. (5Z)-5-(4-Methoxybenzylidene)-[1,3]thiazolo[3,2-b][1,2,4]triazol-6-one (**9**).* Yield 49%, yellow powder, mp 210–212 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4, δ): 8.30 (s, 1H), 8.20 (s, 1H), 7.60 (d, $J = 7.7$ Hz, 2H), 7.10 (d, $J = 7.7$ Hz, 2H), 3.90 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 162.1, 159.2 (C=O), 156.3, 139.7, 133.1, 124.8, 120.9, 115.3, 55.6. Calc. for $C_{12}H_9N_3O_2S$, %: C, 55.59; H, 3.50; N, 16.21. Found, %: C, 55.47; H, 3.62; N, 16.13.

*6.2.2.5. (5Z)-5-(4-Dimethylaminobenzylidene)-[1,3]thiazolo-[3,2-b][1,2,4]triazol-6-one (**10**).* Yield 60%, orange crystals,

mp 259–260 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4, δ): 8.40 (s, 1H), 8.20 (s, 1H), 7.60 (d, $J = 8.0$ Hz, 2H), 6.80 (d, $J = 8.0$ Hz, 2H), 3.60 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 158.8 (C=O), 158.7, 156.5, 152.5, 141.0, 133.5, 118.9, 115.1, 112.2, 39.6. Calc. for $C_{13}H_{12}N_4OS$, %: C, 57.34; H, 4.44; N, 20.57. Found, %: C, 57.22; H, 4.31; N, 20.64.

*6.2.2.6. (5Z)-5-[(2Z)-3-Phenylprop-2-en-1-ylidene]-[1,3]thiazolo[3,2-b][1,2,4]triazol-6-one (**11**).* Yield 60%, brown powder, mp 238–240 °C. ^{1}H NMR (300 MHz, DMSO-d_6 + CCl_4, δ): 8.30 (s, 1H), 8.00 (d, $J = 15.3$ Hz, 1H), 7.60 (d, $J = 8.0$ Hz, 2H), 7.35–7.45 (m, 3H), 7.30 (d, $J = 9.0$ Hz, 1H), 7.00 (dd, $J = 15.3$, 9.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, δ): 158.9 (C=O), 158.8, 147.2, 140.3, 135.2, 130.5, 128.9, 128.4, 125.2, 122.5. Calc. for $C_{13}H_9N_3OS$, %: C, 61.16; H, 3.55; N, 16.46. Found, %: C, 61.07; H, 3.44; N, 16.32.

6.3. Pharmacology

Primary anticancer assay was performed in the 3-cell line panel consisting of NCI-H460 (lung), MCF7 (breast) and SF-268 (CNS), in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda [15–17]. Tested compounds were added to the culture at a single concentration (10^{-4} 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 compound were reported as the percentage 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 compounds which reduced the growth of the cell lines to 32% or less (negative numbers indicate cell kill) were passed on for evaluation in the full panel of nearly 60 human tumor cell lines. The cytotoxic and/or growth inhibitory effects of the reported compounds were tested in vitro against the full panel of 60 human tumor cell lines derived from nine neoplastic diseases 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. For each compound, the 50% growth inhibition (GI_{50}) and total growth inhibition (TGI) were obtained for all the cell lines. Values were calculated for each of these parameters if the level of activity was reached; if the effect was not reached or was exceeded, the value is expressed as greater or lesser than the maximum or minimum concentration tested. The log GI_{50} and log TGI were then determined, defined as the mean of the log's of the individual GI_{50} and TGI values. The lowest values are obtained with the most sensitive cell lines. Compounds having values ≤ 4 were declared to be active. Furthermore, a mean graph midpoints (MG_MID) were calculated for each of the parameters, giving an averaged activity parameter over all cell lines for each compound. For the calculation of the MG_MID, insensitive cell lines are included with the highest concentration tested.

6.4. Crystal structure determination of **10**

Crystal data: $C_{13}H_{12}N_4OS$, monoclinic, space group $P2_1/c$, $a = 11.6122(13)$, $b = 6.8062(9)$, $c = 16.5826(17)$ Å, $\beta = 105.369(9)°$, $V = 1263.7(3)$ Å^3, $Z = 4$, $T = 293(2)$ K.

Data collection: A orange-red lath crystal of $0.60 \times 0.22 \times 0.10$ mm was used to record 4482 (Cu Kα radiation, $\theta_{max} = 70.0°$) intensities on a Kuma KM-4 diffractometer [20]. Accurate unit cell parameters were determined by the least squares refinement fit to the setting angles of 43 reflections collected in range $13.4 \leq \theta \leq 30.3°$. Intensity data collection employed 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 (μ(Cu Kα) = 2.261 mm^{-1}). The minimum and maximum transmissions were 0.491 and 0.798. The 2364 total unique reflections (R(int) = 0.0216) were used for further calculations.

Structure solution and refinement: The structure was solved by the direct methods using SHELXS-97 [21], and refinement was done against F^2 for all data using SHELXL-97. All H atoms were placed in geometrically calculated positions and were refined using a riding model, with 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. The final refinement converged with $R = 0.0371$ (for 2040 data with $F^2 > 4\sigma(F^2)$), $wR = 0.1127$ (on F^2 for all data), and $S = 1.024$ (on F^2 for all data). The largest difference peak and hole were 0.279 and −0.339 eÅ^{-3}. The molecular illustration was drawn using ORTEP-3 for Windows [22]. Software used to prepare material for publication was WINGX [23].

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 607918).

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