Synthesis and anticancer evaluation of novel 3,5-diaryl-thiazolo[4,5-*d*]pyrimidin-2-one derivatives

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Abstract The 2-oxo analogs of thiazolo[4,5-*d*]pyrimidine-2-thiones were prepared to study their cytotoxic activity. Five of the newly synthesized compounds were selected by the National Cancer Institute (Bethesda, MD, USA) for a primary in vitro antitumor assay. 7-Chloro-3,5-diphenyl-thiazolo[4,5-*d*]pyrimidin-2-one (**5a**) proved to be the most active one among the screened derivatives and was further evaluated in the full panel of 60 cell lines at five different concentrations. The structures of compounds were determined by IR, ¹H-NMR, ¹³C-NMR, X-ray, and elemental analysis.

Keywords Antitumor agents · In vitro studies · Thiazolo[4,5-*d*]pyrimidin-2-ones · Synthesis

Introduction

Thiazolo[4,5-*d*]pyrimidines, 7-thio analogs of purines are potentially bioactive molecules. In contrast with related 2-thioxo-thiazolo[4,5-*d*]pyrimidine derivatives, the 2-oxo analogs have not been very well explored in medicinal chemistry. The synthesis and biological evaluation of the substituted 2-oxo-thiazolo[4,5-*d*]pyrimidines have been the subject of several review articles. They were reported to possess antibacterial, antifungal (Akbari *et al.*, 2008; Habib *et al.*, 2007), and anti-inflammatory activity (CXCR2-receptor antagonists) (Walters *et al.*, 2008), inhibit the growth of HCMV-human cytomegalovirus (Revankar *et al.*, 1998), and be corticotrophin-releasing hormone (CRH-R1)

receptor antagonists (display antidepressant activity) (Beck *et al.*, 1999).

In this study, in continuation of our work on thiazolo[4,5-*d*]pyrimidine derivatives, the synthesis and in vitro cytotoxic evaluation of thiazolo[4,5-*d*]pyrimidin-2-ones are reported. These designed thiazolo[4,5-*d*]pyrimidine-2-ones are related to thiazolo[4,5-*d*]pyrimidine-2-thiones that have been previously reported to be potent antitumor agents (Becan and Wagner, 2008). Thiazolo[4,5-*d*]pyrimidine derivatives have been extensively studied as potential drug candidates and also have anticancer activity (Rida *et al.*, 1996; Fahmy *et al.*, 2002, 2003). Most of these compounds provided with anticancer activity possess an aromatic rings and electronegative substituent directly linked to the C-17 of the essential core (Fig. 1) or attached at aromatic moieties. The method involved subsequent treatment of the appropriate 3,5-diaryl-2-thioxo-5,6-dihydro-4*H*-thiazolo[4,5-*d*]pyrimidin-7-ones (**2**) and 7-chloro-3,5-diaryl-thiazolo[4,5-*d*]pyrimidine-2-thiones (**3**) with diethyl sulfate and water for the replacement of the 2-thioxo group by an oxygen function (Scheme 1). Compounds **2** and **3** were obtained from 4-amino-5-carboxamido-3-substituted-2,3-dihydrothiazole-2-thiones (**1**) (Gewald, 1966) according to a reported earlier procedure (Becan and Wagner, 2008). Pyrimidine ring formation with appropriate aryl aldehyde, followed by chlorination provided the desired cores **2** and **3**, bearing the respective aromatic substituent at position 3 and 5, which could further be treated with diethyl sulfate and hydrolyzed to yield 2-thiazolones **4a–4f** and **5a–5f**. All synthesized compounds were submitted to the National Cancer Institute (NCI, Bethesda, Maryland) to evaluate their growth inhibitory effects on 60 human cancer cell lines, derived from nine neoplastic diseases. Five derivatives **4a**, **4b**, **5a**, **5b**, and **5d** were selected for a primary in vitro antitumor assay, at 10^{−5} M concentration. Results were expressed as percent growth of

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Fig. 1 X-Ray molecular structure of compound **5a** with the atom-numbering scheme used in the crystallographic analysis

the treated cells, compound **5a** showing mean percent growth =71.26 was further tested at five different concentrations.

Results and discussion

Chemistry

A series of the new substituted thiazolo[4,5-*d*]pyrimidin-2-ones **4a–4f** and **5a–5f** were synthesized as shown in Scheme 1. The method involved subsequent treatment of the appropriate 3,5-diaryl-2-thioxo-5,6-dihydro-4*H*-thiazolo[4,5-*d*]pyrimidin-7-ones (**2**) and 7-chloro-3,5-diaryl-thiazolo[4,5-*d*]pyrimidine-2-thiones (**3**) (Becan and Wagner, 2008) with diethyl sulfate and water for the replacement of the 2-thioxo group with 2-oxo. First, compounds **2** were obtained through the reaction of the corresponding, refluxing aromatic aldehyde with 4-amino-5-carboxamido-3-substituted-2,3-dihydrothiazole-2-thiones **1** (Gewald, 1966), in the presence of bases according to the earlier reported procedure (Becan and Wagner, 2008). Pyrimidine ring formation with aryl aldehydes followed by chlorination with a mixture of phosphorus pentachloride and phosphorus oxychloride gave the desired cores **2** and **3** which were further treated in boiling acetonitrile with diethyl sulfate. The obtained positively charged 2-ethylthiazolium salt was hydrolyzed to yield thiazolones-2. Yields of reaction were variable and were higher when R1 and R2 were not substituted. Elemental analysis, IR, ^1H and ^{13}C -NMR, and X-ray

data evaluated the structure of synthesized substances. In the IR spectra of compounds **4a–4f**, the two stretching bands of 6-NH group were detected in the range of $3470\text{--}3080\text{ cm}^{-1}$. These compounds showed the characteristic vibrations of the C=O group at $1690\text{--}1670\text{ cm}^{-1}$. In the ^1H -NMR spectra, characteristic signal of compounds **4a–4f** was one-proton singlet of 6 N–H resonated at 13.19–13.27 ppm. Aromatic protons have formed multiplet at 7.22–8.20 ppm.

The formation of chlorination products **5a–5f** was indicated in the IR spectra by the disappearance of stretching bands of 6-NH group. Besides the absorption bands due to C=N and C–S–C functions, the presence of C=O functional group was marked by the appearance of bond ranging from 1690 to 1680 cm^{-1} , which was lacked in the precursor **3**. In the ^1H -NMR spectra of 7-chloro derivatives **5a–5f** we were observed only aromatic protons signal at 7.26–8.22 ppm. The ^{13}C -NMR spectra of the active compounds **5a**, **5b**, and **5d**, given in Table 1, displayed the appropriate number of resonances that exactly fit the number of carbon atoms. The most active compound **5a** was recrystallized from a DMF solvent; the block-shaped crystals formed as a result were submitted to X-ray analysis. Data were collected at 100 K from a single crystal. X-ray crystallography of the most active agent **5a** confirmed the chemical structure (Fig. 1). Crystallographic data for the structure are depicted in Table 2.

Anticancer activity assay

All synthesized compounds were submitted for testing at the NCI to evaluate the growth inhibitory effect. Five compounds **4a**, **4b**, **5a**, **5b**, and **5d** were selected for a primary in vitro antitumor assay (Monks *et al.*, 1991; Boyd and Paull, 1995; Shoemaker *et al.*, 2002). A process beginning with the evaluation of the compound against approximately 60 different human tumor cell lines representing leukemia, melanoma, and cancers of the lung, colon, brain, breast, ovary, prostate, and kidney at 10^{-5} M concentration was performed. With one dose, compound **4b** was devoid of cytotoxic activity (mean growth percent 99.88) and **4a** was slightly active against renal cancer CAKI-1 cell line (26.76 % growth).

Compounds **5a**, **5b**, and **5d** which possess electron-withdrawing 7-chloro substituent showed variable antitumor activity, reported as the percentage of growth of treated cells; the preliminary screening results are shown in Table 3. Compounds **5a**, **5b**, and **5d** exhibited antiproliferative effect against cell lines of leukemia, non-small cell lung cancer, colon cancer, melanoma, ovarian cancer, and renal cancer. It is worth to mention that substance **5a** showed noticeable cytotoxic activity against renal cancer (UO-31), melanoma (MALME-3M), and non-small cell lung cancer (NCI-H522) while compound **5b** was most

Scheme 1 (i) LiOH; (ii) POCl₃/PCl₅; (iii) (C₂H₅)₂SO₄; (iiii) H₂O

effective against the last one. This limited data indicate that the replacement of the 7-oxo group with the small, non-polar chloro substituent substantially increased anticancer activity. Remarkable low growth percent values against a minimum number of cell lines (mean growth) was obtained only for compound **5a** which was approved for the further screening test to evaluate the growth inhibition (GI), and cytostatic and cytotoxic effects. The selected compound was additionally evaluated at tenfold dilution of five different concentrations, from 10⁻⁴ to 10⁻⁸ M on approximately 60 human tumor cell lines panels. Three different dose-response parameters, GI₅₀, TGI, and LC₅₀, were calculated for each cell line. GI₅₀ is the molar concentration of the compound required for half GI. Total growth inhibition (TGI) is the molar concentration of the compound resulting in TGI; TGI signifies the cytostatic effect. LC₅₀ is the molar concentration of the compound resulting in a 50 % death of the initial cells; LC₅₀ signifies the

cytotoxic effect. The overview of these parameters of compound **5a** is reported in Table 4 and compared with log GI₅₀ values of thioguanine (TG), the NCI standard anticancer agent. The log GI₅₀ values lower than -5 showed a notable activity level. It can be noticed that compound **5a** proved to be very sensitive toward non-small cell lung cancer NCI-H522 and renal cancer UO-31 log GI₅₀ -5.91 and -5.88, respectively, (MG_MID: log GI₅₀ -5.1, log TGI -4.4, log LC₅₀ -4.09). GI of most cell lines of standard TG is higher than that showed by investigated compound **5a**; but against the following cell lines: K-562, NCI-H322M, NCI-H522, SW-620, U251, SK-MEL-28, IGROV1, A498, and HS 578T, compound **5a** was more active than TG. TG is a guanine analog and thiazolo[4,5-*d*]pyrimidines can be considered as 7-thio analogs of the purine bases guanine and adenine. Thiazolo[4,5-*d*]pyrimidine derivatives may interfere with the synthesis of guanine nucleotides as antimetabolites.

Table 1 ^{13}C -NMR data of compounds **5a**, **5b**, and **5d**

Comp.	^{13}C -NMR (DMSO- d_6) δ ppm

5a R2=H	168.25 (C15), 166.79 (C5), 160.99 (C7), 157.65 (C17), 150.71 (C4), 135.08 (C6), 133.55 (C16), 133.17 (C1), 132.04 (C10), 131.69 (C13), 129.44 (C9), 129.28 (C11), 129.04 (C2), 128.94 (C3), 128.86 (C12), 128.70 (C14), 128.05 (C8)
5b R2=Cl	168.21 (C15), 166.73 (C5), 159.96 (C17), 157.67 (C7), 155.87 (C4), 150.71 (C6), 136.87 (C16), 136.54 (C1), 133.96 (C10), 133.52 (C3), 133.11 (C12), 130.66 (C13), 129.34 (C9), 129.07 (C14), 129.03 (C8), 128.93 (C11), 128.81 (C2)
5d R2=F	168.21 (C15), 166.75 (C5), 160.04 (C1), 157.59 (C17), 155.64 (C7), 150.71 (C4), 133.49 (C6), 133.11 (C16), 131.60 (C10), 130.50 (C3), 130.38 (C12), 130.19 (C9), 130.07 (C14), 129.16 (C8), 129.30 (C13), 115.97 (C2), 115.76 (C11)

The carbon atom-numbering scheme used in the crystallographic analysis was applied

Table 2 Crystallographic data for compound **5a**

Crystal data and structure refinement	
Empirical formula	$\text{C}_{17}\text{H}_{10}\text{ClN}_3\text{O}_2\text{S}$
Formula weight	339.79
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, Cc
Unit cell dimensions	$a = 11.7588$ (8) Å $\alpha = 90^\circ$ $b = 19.4837$ (14) Å $\beta = 90^\circ$ $c = 7.0758$ (5) Å $\gamma = 90^\circ$
Volume	1468.89 (18) Å ³
Z, calculated density	4, 1.536 Mg/m ³
Absorption coefficient	0.409 mm ⁻¹
F (000)	696
Crystal size	0.20 × 0.10 × 0.10 mm
Theta range for data collection	2.18–27.07°
Limiting indices	$-15 \leq h \leq 15$, $-24 \leq k \leq 24$, $-9 \leq l \leq 9$
Reflection collected/unique	61,281/3,225 [$R(\text{int}) = 0.0320$]
Completeness to theta = 27.07	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min transmission	0.9602 and 0.9226
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3,225/3/208
Goodness-of-fit on F^2	1.036
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0195$, $wR_2 = 0.0520$
R indices (all data)	$R_1 = 0.0197$, $wR_2 = 0.0524$
Absolute structure parameter	−0.02 (3)
Largest diff. peak and hole	0.202 and −0.265 e.Å ⁻³

Table 3 Anticancer activity as growth % in concentration 10^{-5} M for the compounds **5a**, **5b**, and **5d**

Compound	Mean growth%	Range of growth%	Most sensitive panel/cell line growth %
5a	71.26	−84.63 to 124.07	−84.63 Rc/UO-31, −77.98 M/MALME-3M, −69.53 NSCLc/NCI-H522, 3.17 Cc/HCC-2998, 8.46 Cc/HCC-116, 16.05 M/LOX IMVI, 19.57 L/CCRF-CEM, 26.33 L/SR, 33.32 Oc/OVCAR-3
5b	86.17	5.19 to 136.81	5.19 NSCLc/NCI-H522, 21.51 L/SR, 24.35 M/LOX IMVI, 29.34 Cc/HCT-116, 33.63 L/CCRF-CEM, 34.56 L/K-562, 47.57 Cc/SW-620
5d	91.21	−31.63 to 124.32	−31.63 NSCLc/NCI-H522, 28.57 L/SR, 35.79 L/K-562, 40.46 Cc/HCT-116, 41.76 Rc/CAKI-1

Data obtained from the NCIs in vitro disease-oriented human tumor cells

L leukemia, *NSCLc* non-small cell lung cancer, *Cc* colon cancer, *M* melanoma, *Oc* ovarian cancer, *Rc* renal cancer

Experimental

Chemistry

Melting points were determined on a Boethius apparatus and were uncorrected. Elemental analyses for the synthesized compounds were performed on a Perkin Elmer 2400 (Waltham, MA, USA) analyzer, and results within ± 0.4 % of the theoretical values were obtained for the new compounds. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra were acquired in d_6 -DMSO on a Bruker ARX 300 MHz (Bruker Analytic, Karlsruhe, Germany; Bruker AG, Fallanden, Switzerland) instrument. Tetramethylsilane was used as the internal standard and all chemical shift values were expressed in parts per million (δ , ppm). IR spectra were recorded on a Specord M80 spectrometer using KBr pellets. X-Ray Crystallography: the data were collected using the Bruker KAPPA APEXII ULTRA controlled by APEXII software. Reaction progress and the purity of the obtained compounds were monitored by thin-layer chromatography on Merck silica gel plates (Merck F_{254} , Darmstadt, Germany) using the solvent system dichloromethane: 1-propanol (10:1) for elution. Iodine was used as a developing agent. The chemicals and reagents for syntheses were obtained from Alfa Aesar (Karlsruhe, Germany), Chempur (Piekary Sl. Poland), and Sigma-Aldrich (Steinheim, Germany). Starting compounds are synthesized according to the literature (Gewald *et al.*, 1966; Becan and Wagner, 2008).

General procedures for the synthesis of compounds **4a–4f** and **5a–5f**

To a solution of appropriate compound **2** or **3** (10 mmol) in acetonitrile (20 ml), diethyl sulfate (4.62 g, 30 mmol) was added, and the reaction mixture was heated under reflux for 1 h at 130 °C. After cooling, 100 ml of water was added and the reaction mixture was refluxed with stirring for 2 h during which the product was precipitated. The solid was

filtered and suspended in a hot mixture of methanol and 5 % NaHCO_3 . The reaction mixture was allowed to cool, and the crude product was filtered and crystallized from appropriate solvent.

3,5-Diphenyl-6H-thiazolo[4,5-d]pyrimidine-2,7-dione (**4a**)

IR (KBr) cm^{-1} : 3450, 3080 (NH), 1680 (C=O), 1530 (C=N), 1260 (C–S–C), 760 (phenyl). $^1\text{H-NMR}$ (d_6 -DMSO) δ : 7.42–7.93 (m, 10H, arom.), 13.19 (s, 1H, NH). Anal. Calcd for $\text{C}_{17}\text{H}_{11}\text{N}_3\text{O}_2\text{S}$: C, 63.54; H, 3.45; N, 13.08. Found: C, 63.44; H, 3.52; N, 13.27.

5-(4-Chlorophenyl)-3-phenyl-6H-thiazolo[4,5-d]pyrimidine-2,7-dione (**4b**)

IR (KBr) cm^{-1} : 3450, 3090 (NH), 1670 (C=O), 1590 (C=N), 1230 (C–S–C), 760 (phenyl). $^1\text{H-NMR}$ (d_6 -DMSO) δ : 7.51–7.94 (m, 9H, arom.), 13.22 (s, 1H, NH). Anal. Calcd for $\text{C}_{17}\text{H}_{10}\text{ClN}_3\text{O}_2\text{S}$: C, 57.39; H, 2.83; N, 11.81. Found: C, 57.56; H, 3.01; N, 11.97.

5-(2-Chlorophenyl)-3-phenyl-6H-thiazolo[4,5-d]pyrimidine-2,7-dione (**4c**)

IR (KBr) cm^{-1} : 3470, 3080 (NH), 1680 (C=O), 1590 (C=N), 1260 (C–S–C), 760 (phenyl). $^1\text{H-NMR}$ (d_6 -DMSO) δ : 7.34–7.99 (m, 9H, arom.), 13.27 (s, 1H, NH). Anal. Calcd for $\text{C}_{17}\text{H}_{10}\text{ClN}_3\text{O}_2\text{S}$: C, 57.39; H, 2.83; N, 11.81. Found: C, 57.59; H, 2.87; N, 11.85.

5-(4-Fluorophenyl)-3-phenyl-6H-thiazolo[4,5-d]pyrimidine-2,7-dione (**4d**)

IR (KBr) cm^{-1} : 3450, 3090 (NH), 1680 (C=O), 1610 (C=N), 1240 (C–S–C), 770 (phenyl). $^1\text{H-NMR}$ (d_6 -DMSO) δ : 7.31–8.20 (m, 9H, arom.), 13.20 (s, 1H, NH). Anal.

Table 4 The result of the in vitro anticancer activity of compound **5a** against 60 human cancer cell lines

Panel	Cell line	Compound 5a				TG
		Growth (%) ^b 10 ^{−5} M	Log GI ₅₀	Log TGI	Log LC ₅₀	Log GI ₅₀
<i>Leukemia</i>						
	CCRF-CEM	23	−5.42	− ^c	−	−6.5
	HL-60(TB)	38	−5.23	−	−	ns
	K-562	19	−5.39	−	−	−4.0
	MOLT-4	54	−4.89	−	−	ns ^d
	RPMI-8226	75	−4.35	−	−	−5.9
	SR	8	−5.58	−4.67	−	−6.2
<i>Non-Small Cell Lung C.</i>						
	A549/ATCC	87	−4.36	−	−	−5.3
	EKVX	84	−4.59	−	−	−5.4
	HOP-62	15	−5.44	−4.81	−4.14	−6.1
	HOP-92	24	−5.51	−4.17	−	−5.8
	NCI-H23	20	−5.44	−4.51	−	−5.5
	NCI-H322 M	62	−4.85	−4.23	−	−4.6
	NCI-H460	78	−4.60	−	−	−6.0
	NCI-H522	−65	−5.91	−5.52	−	−5.7
<i>Colon C.</i>						
	COLO-205	52	−4.95	−	−	−5.6
	HCC-2998	90	−4.09	−	−	ns
	HCT-116	−53	−5.68	−5.35	−5.02	−6.2
	HCT-15	28	−5.33	−	−	−5.6
	HT29	10	−5.41	−4.72	−	−5.9
	KM12	81	−4.09	−	−	−5.5
	SW620	−4	−5.56	−5.04	−	−5.4
<i>CNS Cancer</i>						
	SF-268	52	−4.98	−4.42	−	−5.9
	SF-295	92	−4.24	−	−	−5.9
	SF-539	52	−4.96	−	−	−6.2
	SNB-19	70	−4.38	−	−	−4.1
	SNB-75	12	−5.73	−4.86	−4.25	−6.0
	U251	20	−5.43	−4.73	−	−5.0
<i>Melanoma</i>						
	LOX IMVI	−44	−5.69	−5.32	−4.74	ns
	MALME-3M	62	−4.83	−4.10	−	−5.5
	M14	16	−5.42	−4.45	−	−6.2
	MDA-MB-435	26	−5.31	−4.34	−	−6.3
	SK-MEL-2	48	−5.04	−4.36	−	−5.8
	SK-MEL-28	9	−5.47	−4.88	−4.16	−5.2
	SK-MEL-5	60	−4.81	−	−	−5.6
	UACC-257	48	−5.05	−4.50	−	−5.2
	UACC-62	62	−4.70	−	−	−6.4
<i>Ovarian C.</i>						
	IGROV1	−65	−5.75	−5.32	−4.74	−5.2
	OVCAR-3	−41	−5.75	−4.10	−	−5.8
	OVCAR-4	31	−5.30	−4.45	−	−5.3
	OVCAR-5	90	−	−4.34	−	−6.3

Table 4 continued

Panel Cell line	Compound 5a				TG
	Growth (%) ^b 10 ^{−5} M	Log GI ₅₀	Log TGI	Log LC ₅₀	Log GI ₅₀
OVCAR-8	−45	−5.69	−4.36	—	−6.4
NCI/ADR-RES	66	−4.67	—	—	−6.4
SK-OV-3	81	—	—	—	−6.3
<i>Renal Cancer</i>					
786-0	41	−5.15	−4.25	—	−5.8
A498	44	−5.46	—	—	−4.6
ACHN	42	−5.16	—	—	−5.4
CAKI-1	−30	−5.63	−5.24	−4.33	−6.5
SN12C	43	−5.13	—	—	−5.1
TK-10	51	−4.98	—	—	−6.3
UO-31	−79	−5.88	−5.54	—	−6.1
RXF 393	−4	−5.62	−5.05	−4.42	−6.3
<i>Prostate C.</i>					
PC-3	11	−5.48	−4.84	−4.09	−5.5
DU-145	34	−5.33	−4.63	−4.09	−6.3
<i>Breast C.</i>					
MCF7	77	−4.19	—	—	−6.3
MDA-MB-231/ATCC	37	−5.20	—	—	ns
HS 578T	12	−5.48	−4.73	—	−5.2
BT-549	86	—	—	—	−5.9
T-47D	57	−4.77	—	—	−5.0
MDA-MB-468	20	−5.44	—	—	ns
MG_MID ^c		−5.1	−4.4	−4.09	

^a Data obtained from the NCI's in vitro disease-oriented human tumor cells^b Values greater than zero mean percentage of growth and those less than zero mean percentage of lethality to the tumor cell line^c The values greater than −4 were excluded^d Cell line not screened^e MG_MID (mean graph midpoint) arithmetical mean value for all tested cell lines

Calcd for C₁₇H₁₀FN₃O₂S: C, 60.17; H, 2.97; N, 12.38.
 Found: C, 59.98; H, 3.03; N, 12.41.

3,5-Bis(4-fluorophenyl)-6H-thiazolo[4,5-d]pyrimidine-2,7-dione (4e)

IR (KBr) cm^{−1}: 3470, 3090 (NH), 1690 (C=O), 1570 (C=N), 1240 (C–S–C), 780 (phenyl). ¹H-NMR (*d*₆-DMSO) δ: 7.22–8.03 (m, 8H, arom.), 13.21 (s, 1H, NH). Anal. Calcd for C₁₇H₉FN₃O₂S: C, 57.14; H, 2.54; N, 11.76. Found: C, 57.31; H, 2.55; N, 11.94.

3-(4-Bromophenyl)-5-phenyl-6H-thiazolo[4,5-d]pyrimidine-2,7-dione (4f)

IR (KBr) cm^{−1}: 3450, 3080 (NH), 1680 (C=O), 1590 (C=N), 1260 (C–S–C), 760 (phenyl). ¹H-NMR (*d*₆-DMSO) δ: 7.45–8.16 (m, 9H, arom.), 13.19 (s, 1H, NH). Anal.

Calcd for C₁₇H₁₀BrN₃O₂S: C, 51.01; H, 2.52; N, 10.50.
 Found: C, 51.14; H, 2.60; N, 10.66.

7-Chloro-3,5-diphenyl-thiazolo[4,5-d]pyrimidin-2-one (5a)

IR (KBr) cm^{−1}: 1680 (C=O), 1590 (C=N), 1260 (C–S–C), 760 (phenyl). ¹H-NMR (*d*₆-DMSO) δ: 7.46–8.13 (m, 10H, arom.). Anal. Calcd for C₁₇H₁₀ClN₃OS: C, 60.09; H, 2.97; N, 12.37. Found: C, 59.98; H, 3.01; N, 12.38.

7-Chloro-5-(4-chlorophenyl)-3-phenyl-thiazolo[4,5-d]pyrimidin-2-one (5b)

IR (KBr) cm^{−1}: 1680 (C=O), 1560 (C=N), 1230 (C–S–C), 760 (phenyl). ¹H-NMR (*d*₆-DMSO) δ: 7.52–8.11 (m, 9H, arom.). Anal. Calcd for C₁₇H₉Cl₂N₃OS: C, 54.56; H, 2.42; N, 11.23. Found: C, 54.60; H, 2.49; N, 11.29.

Table 5 Characterization data of compounds **4a–4f** and **5a–5f**

Compound	R1	R2	m.p. (°C)	Yield %	Molecular formula	Molecular weight	Solvent
4a	–	–	279–280	64.55	C ₁₇ H ₁₁ N ₃ O ₂ S	321,35	1-Propanol
4b	–	4-Cl	369–370	58.77	C ₁₇ H ₁₀ ClN ₃ O ₂ S	355,81	DMF-1-propanol 3:1
4c	–	2-Cl	338–339	58.88	C ₁₇ H ₁₀ ClN ₃ O ₂ S	355,81	DMF-1-propanol 3:1
4d	–	4-F	348–349	56.51	C ₁₇ H ₁₀ FN ₃ O ₂ S	339,31	DMF-water
4e	4-F	4-F	327–328	49.73	C ₁₇ H ₉ F ₂ N ₃ O ₂ S	357,33	1-Propanol
4f	4-Br	–	210–211	55.77	C ₁₇ H ₁₀ BrN ₃ O ₂ S	400,25	DMF-1-propanol 3:1
5a	–	–	194–195	63.87	C ₁₇ H ₁₀ ClN ₃ OS	339,80	DMF-1-propanol 3:1
5b	–	4-Cl	171–172	57.45	C ₁₇ H ₉ Cl ₂ N ₃ OS	374, 24	DMF-water
5c	–	2-Cl	240–241	52.32	C ₁₇ H ₉ Cl ₂ N ₃ OS	374,24	DMF-1-propanol 3:1
5d	–	4-F	235–236	54.73	C ₁₇ H ₉ ClFN ₃ OS	357,79	DMF-1-propanol 3:1
5e	4-F	4-F	234–235	48.67	C ₁₇ H ₈ ClF ₂ N ₃ OS	375,78	DMF-1-propanol 3:1
5f	4-Br	–	388–390	57.46	C ₁₇ H ₉ BrClN ₃ OS	418,69	DMF-1-propanol 3:1

*7-Chloro-5-(2-chlorophenyl)-3-phenyl-thiazolo
[4,5-d]pyrimidin-2-one (5c)*

IR (KBr) cm^{−1}: 1690 (C=O), 1570 (C=N), 1250 (C–S–C), 760 (phenyl). ¹H-NMR (*d*₆-DMSO) δ: 7.52–8.11 (m, 9H, arom.). Anal. Calcd for C₁₇H₉Cl₂N₃OS: C, 54.56; H, 2.42; N, 11.23. Found: C, 54.65; H, 2.50; N, 11.33.

*7-Chloro-5-(4-fluorophenyl)-3-phenyl-thiazolo
[4,5-d]pyrimidin-2-one (5d)*

IR (KBr) cm^{−1}: 1690 (C=O), 1600 (C=N), 1240 (C–S–C), 760 (phenyl). ¹H-NMR (*d*₆-DMSO) δ: 7.27–8.15 (m, 9H, arom.). Anal. Calcd for C₁₇H₉ClN₃OS: C, 57.10; H, 2.73; N, 11.75. Found: C, 57.21; H, 2.86; N, 11.83.

7-Chloro-3,5-bis(4-fluorophenyl)thiazolo[4,5-d]pyrimidin-2-one (5e)

IR (KBr) cm^{−1}: 1690 (C = O), 1590 (C = N), 1250 (C–S–C), 770 (phenyl). ¹H-NMR (*d*₆-DMSO) δ: 7.26–8.22 (m, 8H, arom.). Anal. Calcd for C₁₇H₈ClF₂N₃OS: C, 54.34; H, 2.15; N, 11.18. Found: C, 54.42; H, 2.20; N, 11.26.

*3-(4-Bromophenyl)-7-chloro-5-phenyl-thiazolo[4,5-
d]pyrimidin-2-one (5f)*

IR (KBr) cm^{−1}: 1680 (C=O), 1560 (C=N), 1250 (C–S–C), 770 (phenyl). ¹H-NMR (*d*₆-DMSO) δ: 7.33–8.16 (m, 8H, arom.). Anal. Calcd for C₁₇H₉BrClN₃OS: C, 48.77; H, 2.17; N, 10.04. Found: C, 48.91; H, 2.25; N, 10.12.

Characteristic data of the new compounds are depicted in Table 5.

Antitumor in vitro screening

The antitumor studies were performed at the NCI (Bethesda, MD, USA). The test agents were preliminarily evaluated in the full panel of 60 different human tumor cell lines derived from nine cancer diseases, namely leukemia, melanoma, non-small cell lung c., colon c., brain c., breast c., ovarian c., prostate c., and renal c., at single 10^{−5} M concentration. Compounds with significant GI are evaluated at five different concentrations ranging from 10^{−4} to 10^{−8} M. The percent growth was evaluated versus controls not treated with tested compounds. Preparation of the tested compounds and the sulforhodamine B (SRB) protein assay which was used to estimate cell viability of growth were described previously (Becan and Wagner, 2008; Monks *et al.*, 1991; Boyd and Paull, 1995; Shoemaker *et al.*, 2002).

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