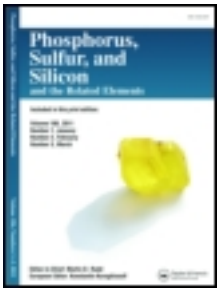

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Synthesis and Anticancer Activity of Novel Nonfused Bicyclic Thiazolidinone Derivatives

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Synthesis and Anticancer Activity of Novel Nonfused Bicyclic Thiazolidinone Derivatives

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*A series of new 2-{4-oxo-2-[(4-oxothiazolidin-2-ylidene)-hydrazono]-thiazolidin-5-yl}-N-arylacetamides (**4a–e**), 5-(2-oxo-2-aryl-ethyl)-2-[(4-oxothiazolidin-2-ylidene)-hydrazono]-thiazolidine-4-ones (**5a–d**), 2-4-oxo-2-[(2-oxothiazolidin-4-ylidene)-hydrazono]-thiazolidin-5-yl-N-arylacetamides (**7a–e**), and 5-(2-oxo-2-aryl-ethyl)-2-[(2-oxothiazolidin-4-ylidene)-hydrazono]-thiazolidine-4-ones (**8a–d**) have been synthesized starting from 2-thioxothiazolidin-4-one and 4-thioxothiazolidin-2-one through a multistep reaction sequence. 2-Thioxothiazolidin-4-one was alkylated via the intermediate formation of the triethylammonium salt **1** by ethyl chloroacetate. Compound **2** and 4-thioxothiazolidin-2-one reacted with thiosemicarbazides to give the 1-(4-thiazolidinone-2-ylidene)-4-R-thiosemicarbazones (**3a,b**) and 1-(2-thiazolidinone-4-ylidene)thiosemicarbazones (**6a,b**), respectively. Following [2+3]-cyclization of thiazolidinone-substituted thiosemicarbazones (**3a,b** and **6a,b**) with N-arylmaleimides and aroylacrylic acids as equivalents of dielectrophilic synthon $[C_2]^{2+}$, novel non-fused bicyclic thiazolidinones (**4a–e, 5a–d, 7a–e, 8a–d**) were synthesized. The structures of the new compounds (**4a–e, 5a–d, 7a–e, 8a–d**) were established on the basis of their elemental analysis and ^1H NMR and mass spectral data. Eight of the synthesized compounds were tested, and three of them displayed different levels of antitumor activity. The most efficient antitumor agent—2-{4-oxo-3-furylmethyl-2-[(4-oxothiazolidin-2-ylidene)-hydrazono]-thiazolidin-5-yl}-N-4-chlorophenylacetamide (**4d**) was found to be active against leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancer cell lines with mean $lgGI_{50}$ and lgTGI values of –5.35 and –4.78, respectively.*

Keywords Anticancer activity; [2+3]-cyclization; thiazolidinones

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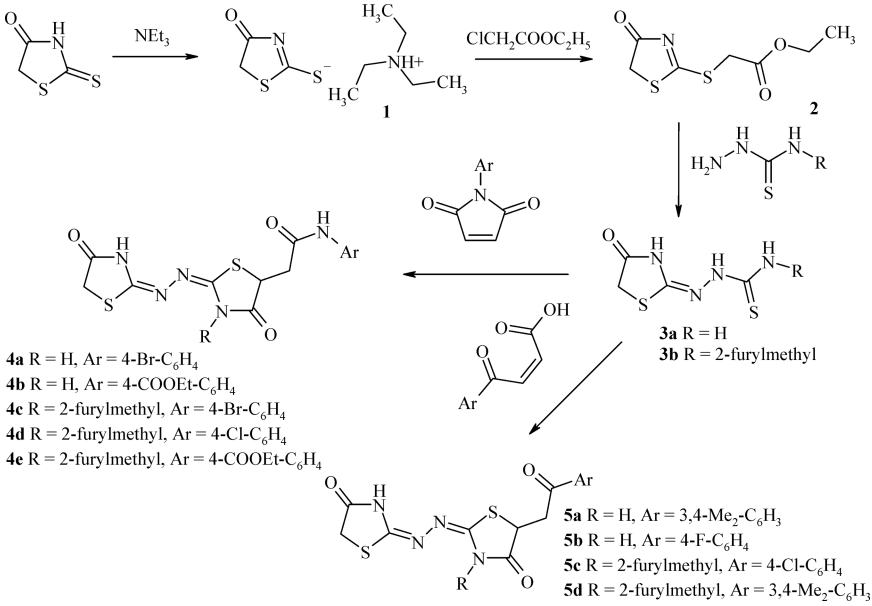
INTRODUCTION

Thiazolidinone derivatives are a traditionally known class of biologically active compounds. In recent years, a large number of innovative drugs containing the thiazolidinone moiety have been developed, including hypoglycemic thiazolidinediones (pioglitazone and its analogs), dual COX-2/5-LOX inhibitors (darbufelon), new generation diuretics (etozolin), etc.[1] Using modern technologies such as virtual and high-throughput screening, combinatorial chemistry, and molecular modeling, it was established that 4-thiazolidinones possess a high affinity to the PPAR-receptors family and are selective inhibitors of UDP-MurNAc/L-Ala ligase.[2–6] As a consequence, a series of potential anti-inflammatory, hypoglycemic, cardiovascular, and antimicrobial agents at different stages of clinical studies were created. Advanced studies of PPARγ-receptors stimulated the concept that PPARγ agonists, including thiazolidinediones, have therapeutic potential in treatment of some cancer types. Moreover, binding inhibitors of antiapoptic proteins Bcl-X_L and BH3[7] which promote normalization of natural cell death and inhibitors of TNF-α binding to TNFRc-1,[8] as well as inhibitors of translation initiation, which cause cell cycle arrest in G1 phase via partial depletion of intercellular Ca^{2+} stores, were identified among 4-thiazolidinones.[9] In this article, we enlarge the database of anticancer thiazolidinones and report on the synthesis of a series of novel nonfused bicyclic thiazolidinones.

RESULTS AND DISCUSSION

The general methods for the synthesis of the new bicyclic nonfused thiazolidinones are depicted in Schemes 1 and 2. As starting reagents, several thiazolidinone-substituted thiosemicarbazones were used.

Following a procedure from the literature, we prepared 1-(4-thiazolidinon-2-ylidene)thiosemicarbazones **3** by prolonged heating of equimolar amounts of 2-thioxo-4-thiazolidinone (rhodanine) and thiosemicarabazide in ethanol.[10] In order to improve this method and enhance the reactivity of rhodanine, the latter was alkylated via the intermediate formation of the triethylammonium salt **1** by ethyl chloroacetate in refluxing acetone, as reported.[11] On reaction of the resulting 2-carbethoxymethylthio-2-thiazolin-4-one **2** with thiosemicarbazide or 4-(2-furylmethyl)thiosemicarbazide in refluxing ethanol, the 1-(4-thiazolidinon-2-ylidene)-4-*R*-thiosemicarbazones (**3a, b**) were obtained (Scheme 1). The isomeric 1-(2-thiazolidinon-4-ylidene)thiosemicarbazones (**6a, b**) were synthesized by the reaction



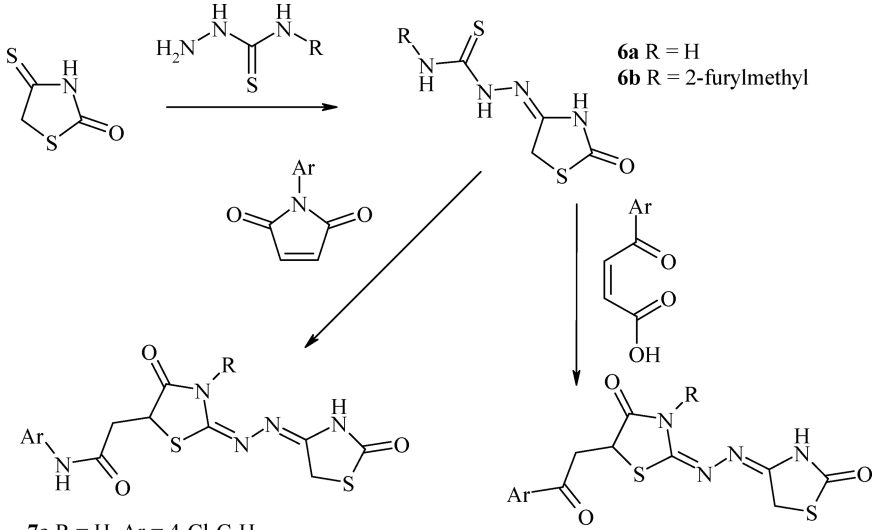
SCHEME 1

of 4-thioxo-2-thiazolidinone (isorhodanine) with the same thiosemicarbazides in refluxing ethanol (Scheme 2).

In order to prepare the bicyclic thiazolidinones, we followed a route to the 4-thiazolidinone moiety, which is based on the [2+3]-cyclization of thiazolidinone-substituted thiosemicarbazones with equivalents of the dielectrophilic synthon $[C_2]$.[2+] We studied the interaction of the above-mentioned binucleophiles with *N*-arylmaleimides and aroylacrylic acids. As a result, a group of new isomeric bicyclic thiazolidinones have been synthesized for a detailed elaboration of a structure–anticancer activity relationship (Schemes 1 and 2).

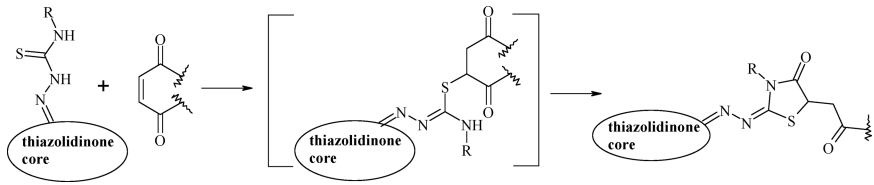
The [2+3]-cyclization of the thiazolidinone-substituted thiosemicarbazone with one equivalent of the dielectrophilic synthon $[C_2]^{2+}$ consists of two steps: 1) addition of the thiosemicarbazone isoform to the double bond of the *N*-arylmaleimide or aroylacrylic acid, and 2) a spontaneous cyclization of intermediate to form the thiazolidine ring (Scheme 3).

The characteristic data of the new nonfused bicyclic thiazolidinones are given in the Experimental section. The analytical and spectral data (^{1}H NMR, EI-MS) confirm the structure and the purity of the synthesized compounds.



SCHEME 2

In the ^{1}H NMR spectra of synthesized compounds, the protons of the CH_2-CH moiety show the characteristic pattern of an AMX spin system.[12] The chemical shifts of the protons H_A, H_M, and H_X were determined to $\delta = 2.85$–3.61, $\delta = 3.13$–4.05, and $\delta = 4.25$–4.44, respectively, with coupling constants $J_{AM} = 16.7$–18.9, $J_{AX} = 8.4$–10.8, and $J_{MX} = 2.4$–4.4 Hz. The protons of the methylene group in **4–5** and **7–8** appear at $\delta = 3.72$–4.32. The ^{1}H NMR spectra of the nonfused bicyclic thiazolidinones show singlets at $\delta = 10.12$–10.48, $\delta = 11.32$–11.76, and $\delta = 11.69$–11.80 corresponding to the NH protons. The signals of the protons of the furyl fragment in **4c–4e, 5c–5d, 7c–7e,** and **8c–8d** appear at $\delta = 6.31$–6.34, $\delta = 6.39$–6.45, and $\delta = 7.22$–7.45. In the ^{1}H NMR



SCHEME 3

TABLE I Anticancer Screening Data of 60 Cancer Cell Line Assays in Concentration 10^{-5}M

	60 cell lines assay in 1 dose 10^{-5}M conc.				
	Mean growth%	Range of growth%	The most sensitive cell line	Growth% of the most sensitive sensitive cell line	Active (selected for 5-dose 60 cell line assays)
4d	14.45	–62.88 to 62.81	SK-MEL-5 (Melanoma)	–62.88	A
7d	88.08	32.82 to 127.32	SR (Leukemia)	32.82	N
7e	102.11	69.49 to 147.39	UO-31 (Renal cancer)	69.49	N
8b	102.98	14.68 to 133.49	UACC-257 (Melanoma)	14.68	N
8c	99.03	74.50 to 123.67	HOP-92 (Non-small cell lung cancer)	74.50	N
8d	96.69	34.93 to 154.34	CAKI-1 (Renal cancer)	34.93	N

spectra of **7c–7e** and **8c–8d**, the protons of the CH_2-CH moiety show three multiplets at $\delta = 2.85$–3.61, $\delta = 3.13$–4.05, and $\delta = 4.25$–4.44. For the NH protons of the thiazolidinone ring, two singlets at $\delta = 11.32$–11.38 and $\delta = 11.52$–11.75, and for the amide protons of **7c–7e** two signals at $\delta = 10.12$–10.31 are observed. This can be explained by the presence of *syn/anti* isomers of these derivatives.

Evaluation of the Anticancer Activity In Vitro

The activity of some of the new nonfused bicyclic thiazolidinones (**4d, 7d, 7c, 8b–8d**) at a single concentration of 10^{-5} M against 57 cancer cell lines was evaluated. 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, and described elsewhere.[13–16] 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. Compound **4d** showed considerable activity and was selected for an advanced assay against full panel (57 cell lines) at five concentrations. On the other hand, the isomer **7d** showed only moderate cytotoxicity. The results of this screening are presented in Table I.

Finally, compound **4d**, as well as compounds **4b** and **7a** without preliminary screening, were tested in vitro against a full panel of about 60 tumor 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 and used at tenfold dilutions of five concentrations (100 μM, 10 μM, 1 μM, 0.1 μM, and 0.01 μM).[13–16] The percentage of growth was evaluated spectrophotometrically versus control cells 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 each experimental agent 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. Compounds having values of –4.00 and less were declared to be active. Values were calculated for each of these parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value was expressed as greater or less than the maximum or minimum concentration tested. Furthermore, mean graph midpoints (MG_MID) were calculated for each of the parameters, giving an average activity parameter over all cell lines for each compound. For the calculation of the MG_MID, insensitive cell lines are included with the highest concentration tested. The results of five concentrations' full panel screening are summarized in Table II.

TABLE II Summary of Anticancer Screening Data at 60 Cancer Cell Lines Dose–Dependent Assay

		Log GI_{50}			Log TGI			Log LC_{50}		
	N[a]	N1[b]	Range	MG_MID	N2[b]	Range	MG_MID	N3[b]	Range	MG_MID
4b	57	5	–5.41 to –4.00	–4.05	1	–4.80 to –4.00	–4.01	0	–	–4.00
4d	56	56	–5.94 to –4.36	–5.35	55	–5.42 to –4.00	–4.78	50	–4.81 to –4.00	–4.29
7a	57	2	–5.61 to –4.00	–4.04	1	–4.55 to –4.00	–4.01	0	–	–4.00

[a]N – Number of human tumor cell lines tested at the 2nd stage assay.

[b]N1, N2, N3 – Number of sensitive cell lines, against which the compound possessed considerable growth inhibition according to the mentioned parameter (Parameter Log < –4.00).

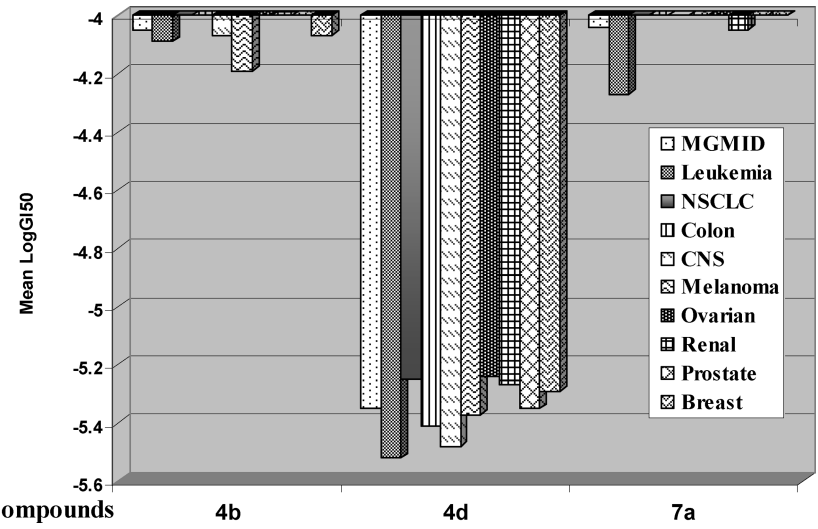


FIGURE 1 Anticancer selectivity pattern of the most active compounds **4b**, **4d**, and **7a**.

Compound **4d** showed the highest cytotoxicity and was active against all tested human tumour cell lines (Table II). Interestingly, the isomeric compound **7d** is inactive to most of the tested cancer cell lines. Selectivity pattern analysis of cell lines by disease origin can definitely affirm selective action of compound **4b** on melanoma cell lines and of compound **7a** on leukemia (Figure 1). These compounds appeared to be the most active against selected individual cell lines with $\log GI_{50}$ varying from –5.61 to –5.41 (Table III). Compound **4b** was found to be a highly active growth inhibitor of the melanoma cell line M14 ($\log GI_{50} = -5.41$), and compound **7b** showed significant activity against the leukemia cell line CCRF-CEM ($\log GI_{50} = -5.61$).

EXPERIMENTAL

The starting compounds 2-thioxo-4-thiazolidinone,[17] 4-thioxo-2-thiazolidinone,[18] and 2-carbethoxymethylthio-2-thiazolin-4-one[11] were obtained according to methods described previously.

Melting points were measured in open capillary tubes on a BÜCHI B-545 melting point apparatus and are uncorrected. The elemental analyses (C, H, N) were performed using a Perkin-Elmer 2400 CHN analyzer and were within ± 0.4% of the theoretical values. The ^{1}H-NMR spectra were recorded on Varian Gemini spectrometer at

TABLE III The Most Sensitive to the Synthesized Compounds Individual Tumor Cell Lines (log $GI_{50} \leq -5.40$)

Compound	Disease	Cell line	logGI_{50}	logTGI
4b	Melanoma	M14	–5.41	–4.80
4d	Leukemia	CCRF-CEM	–5.49	–4.95
	Leukemia	HL-60(TB)	–5.68	–5.28
	Leukemia	K-562	–5.50	–4.90
	Leukemia	RPMI-8226	–5.58	–5.07
	NSC lung cancer	NCI-H460	–5.47	–4.92
	NSC lung cancer	NCI-H522	–5.45	–4.81
	Colon cancer	COLO 205	–5.44	–4.90
	Colon cancer	HCT-116	–5.41	–4.80
	Colon cancer	HCT-15	–5.74	–5.44
	Colon cancer	HT29	–5.62	–5.14
	Colon cancer	KM 12	–5.60	–4.80
	Colon cancer	SW-620	–5.42	–4.87
	CNS cancer	SF-295	–5.59	–4.98
	CNS cancer	SF-539	–5.58	–5.03
	CNS cancer	SNB-75	–5.75	–5.19
	Melanoma	MALME-3M	–5.44	–4.79
	Melanoma	M14	–5.43	–4.82
	Melanoma	SK-MEL-28	–5.41	–4.66
	Melanoma	SK-MEL-5	–5.56	–4.87
	Melanoma	UACC-62	–5.46	–4.80
	Ovarian cancer	OVCAR-3	–5.49	–4.88
	Ovarian cancer	OVCAR-4	–5.49	–4.74
	Renal cancer	A498	–5.44	–4.82
	Renal cancer	RXF 393	–5.66	–5.07
	Breast cancer	MCF7	–5.58	–4.84
	Breast cancer	MDA-MB-435	–5.94	–5.42
	Breast cancer	T-47D	–5.48	–4.77
7a	Leukemia	CCRF-CEM	–5.61	–4.55

300 MHz using a mixture of DMSO-d_6 + CCl_4 as a solvent and TMS as an internal standard. Chemical shifts are reported in ppm. EI-MS were obtained on Varian 1200 L instrument.

Synthesis of 1-(4-Thiazolidinone-2-ylidene)-4-*R*-thiosemicarbazones (3a, b)

A mixture of 2-carbethoxymethylthio-2-thiazolin-4-one (20 mmol) and thiosemicarbazide or 4-(2-furylmethyl)thiosemicarbazide (20 mmol) were refluxed for 0.5 h in 150 mL of ethanol. After cooling to room temperature, a light violet powder precipitated, which was filtered off,

washed with 50 mL of methanol, and recrystallized from a DMF:ethanol mixture (1:2).

3a: Violet crystals; yield 2.85 g (75%); mp 198–199°C (lit. 196–197°C[10]); Anal. Calcd. for $C_4H_6N_4OS_2$: C, 25.25; H, 3.18; N, 29.45%. Found: C, 25.40; H, 3.30; N, 29.50%.

3b: Violet crystals; yield 4.23 g (78%); mp 184–185°C; Anal. Calcd. for $C_9H_{10}N_4O_2S_2$: C, 39.99; H, 3.73; N, 20.73%. Found: C, 40.12; H, 3.85; N, 20.61%.

Synthesis of 1-(2-Thiazolidinon-4-ylidene)-4-*R*-thiosemicarbazones (6a, b)

To a solution of 4-thioxothiazolidin-2-one (20 mmol) in ethanol (150 mL), thiosemicarbazide (20 mmol) in water (20 mL) or 4-(2-furylmethyl)thiosemicarbazide (20 mmol) in ethanol (20 mL) was added. The reaction mixture was refluxed for 0.5 h. After cooling to room temperature, a light brown powder precipitated, which was filtered off, washed with 50 mL of methanol, and recrystallized from a DMF:ethanol mixture (1:2).

6a: Brown crystals; yield 2.75 g (72%); mp 191–192°C (lit. 192°C [19]); Anal. Calcd. for $C_4H_6N_4OS_2$: C, 25.25; H, 3.18; N, 29.45%. Found: C, 25.12; H, 3.32; N, 29.31%.

6b: Brown crystals; yield 3.47 g (64%); mp 171–172°C; Anal. Calcd. for $C_9H_{10}N_4O_2S_2$: C, 39.99; H, 3.73; N, 20.73%. Found: C, 39.86; H, 3.61; N, 20.84%.

Synthesis of 2-4-Oxo-2-[(4-oxothiazolidin-2-ylidene)-hydrazono]-thiazolidin-5-yl-*N*-arylacetamides (4a–e), 5-(2-Oxo-2-aryl-ethyl)-2-[(4-oxothiazolidin-2-ylidene)-hydrazono]-thiazolidin-4-ones (5a–d), 2-{4-Oxo-2-[(2-oxothiazolidin-4-ylidene)-hydrazono]-thiazolidin-5-yl}-*N*-arylacetamides (7a–e), and 5-(2-Oxo-2-aryl-ethyl)-2-[(2-oxothiazolidin-4-ylidene)-hydrazono]-thiazolidin-4-ones (8a–d): General Procedure

A mixture of the appropriate thiazolidinone-substituted thiosemicarbazone (**3a, b, 6a, b**) (5 mmol) and the respective *N*-arylmaleimide (5 mmol) or β-aroylacrylic acid (5 mmol) was refluxed for 1 h in 10 mL of acetic acid. After cooling to room temperature, the precipitated powder was filtered off, washed with 10 mL of water and 10 mL of methanol, and recrystallized from a DMF:ethanol mixture (1:2).

4a: Pink crystals; yield 1.52 g (69%); mp >240°C; ^{1}H NMR (300 MHz, DMSO-d_6+ CCl_4): $\delta = 2.88$ (dd, $J = 18.0$, 9.8 Hz, 1H), 3.13 (dd, $J = 18.0$, 2.9 Hz, 1H), 3.77 (s, 2H), 4.28 (dd, $J = 9.8$, 2.9 Hz, 1H), 7.39 (d, $J = 8.8$ Hz, 2H), 7.51 (d, $J = 8.8$ Hz, 2H), 10.19 (s, 1H), 11.76 (s, 1H), 11.80 (s, 1H); Anal. Calcd. for $C_{14}H_{12}$B3àr$N_5O_3S_2$: C, 38.02; H, 2.73; N, 15.83%. Found: C, 38.20; H, 2.60; N, 15.90%.

4b: Pink crystals; yield 1.41 g (65%); mp 278–280°C; ^{1}H NMR (300 MHz, DMSO-d_6+ CCl_4): $\delta = 1.31$ (t, $J = 6.9$ Hz, 3H), 2.98 (dd, $J = 17.8$, 10.0 Hz, 1H), 3.22 (dd, $J = 17.8$, 3.4 Hz, 1H), 3.86 (s, 2H), 4.28 (q, $J =$ 6.9 Hz, 2H), 4.29 (dd, $J = 10.0$, 3.4 Hz, 1H), 7.89 (d, $J = 8.8$ Hz, 2H), 7.91 (d, $J = 8.8$ Hz, 2H), 10.48 (s, 1H), 11.74 (s, 1H), 11.82 (s, 1H); EI-MS (m/z): 435 (M^+); Anal. Calcd. for $C_{17}H_{17}N_5O_5S_2$: C, 46.89; H, 3.93; N, 16.08%. Found: C, 46.70; H, 3.80; N, 15.90%.

4c: Pink crystals; yield 1.77 g (68%); mp >240°C; ^{1}H NMR (300 MHz, DMSO-d_6+ CCl_4): $\delta = 2.85$ (dd, $J = 17.0$, 10.2 Hz, 1H), 3.28 (dd, $J =$ 17.0, 3.2 Hz, 1H), 3.74 (s, 2H), 4.39 (dd, $J = 10.2$, 3.2 Hz, 1H), 4.88 (s, 2H) 6.32 (br s, 1H), 6.42 (br s, 1H), 7.41 (d, $J = 8.8$ Hz, 2H), 7.42 (br s, 1H), 7.52 (d, $J = 8.8$ Hz, 2H), 10.12 (s, 1H), 11.75 (s, 1H); EI-MS (m/z): 523 (M^+); Anal. Calcd. for $C_{19}H_{16}BrN_5O_4S_2$: C, 43.69; H, 3.09; N, 13.41%. Found: C, 43.58; H, 2.95; N, 13.54%.

4d: Pink crystals; yield 1.33 g (55%); mp >240°C; ^{1}H NMR (300 MHz, DMSO-d_6+ CCl_4): $\delta = 2.85$ (dd, $J = 16.8$, 10.1 Hz, 1H), 3.26 (dd, $J =$ 16.8, 3.1 Hz, 1H), 3.72 (s, 2H), 4.37 (dd, $J = 10.1$, 3.1 Hz, 1H), 4,89 (s, 2H), 6.33 (br s, 1H), 6.45 (br s, 1H), 7.22 (d, $J = 8.6$ Hz, 2H), 7.42 (br s, 1H), 7.56 (d, $J = 8.6$ Hz, 2H), 10.13 (s, 1H), 11.73 (s, 1H); Anal. Calcd. for $C_{19}H_{16}ClN_5O_4S_2$: C, 47.75; H, 3.37; N, 14.65%. Found: C, 47.63; H, 3.51; N, 14.77%.

4e: Pink crystals; yield 1.57 g (61%); mp >240°C; ^{1}H NMR (300 MHz, DMSO-d_6+ CCl_4): $\delta = 1.33$ (t, $J = 7.0$ Hz, 3H), 2.88 (dd, $J = 16.7$, 9.9 Hz, 1H), 3.29 (dd, $J = 16.7$, 3.3 Hz, 1H), 3.75 (s, 2H), 4.27 (q, $J =$ 7.0 Hz, 2H), 4.41 (dd, $J = 9.9$, 3.3 Hz, 1H), 4,87 (s, 2H), 6.33 (br s, 1H), 6.43 (br s, 1H), 7.42 (br s, 1H), 7.65 (d, $J = 8.6$ Hz, 2H), 7.87 (d, $J = 8.6$ Hz, 2H), 10.33 (s, 1H), 11.75 (s, 1H); Anal. Calcd. for $C_{22}H_{21}N_5O_6S_2$: C, 51.25; H, 4.11; N, 13.58%. Found: C, 51.17; H, 4.23; N, 13.71%.

5a: Pink crystals; yield 1.20 g (64%); mp >250°C; ^{1}H NMR (300 MHz, DMSO-d_6+ CCl_4): $\delta = 2{,}32$ (s, 6H), 3.48 (dd, $J = 18.6$, 10.8 Hz, 1H), 3.86 (dd, $J = 18.6$, 2.9 Hz, 1H), 3.89 (s, 2H), 4.25 (dd, $J = 10.8$, 2.9 Hz, 1H), 7.22 (d, $J = 7.6$ Hz, 1H), 7.67 (d, $J = 7.8$ Hz, 1H), 7.72 (s, 1H), 11.65 (s, 1H), 11.73 (s, 1H); Anal. Calcd. For $C_{16}H_{16}N_4O_3S_2$: C, 51.05; H, 4.28; N, 14.88%. Found: C, 51.18; H, 4.31; N, 14.76%.

5b: Pink crystals; yield 1.30 g (71%); mp >250°C; ^{1}H NMR (300 MHz, DMSO-d_6+ CCl_4): $\delta = 3.55$ (dd, $J = 18.8$, 10.4 Hz, 1H), 3.73 (s, 2H), 3.92

(dd, $J = 18.8$, 2.8 Hz, 1H), 4.28 (dd, $J = 10.4$, 2.8 Hz, 1H), 7.23 (t, $J =$ 8.5 Hz, 2H), 8.06 (m, 1H), 11.66 (s, 1H), 11.75 (s, 1H); Anal. Calcd. for $C_{14}H_{11}FN_4O_3S_2$: C, 45.89; H, 3.03; N, 15.29%. Found: C, 45.98; H, 3.21; N, 15.41%.

5c: Pink crystals; yield 1.36 g (59%); mp >240°C; ^{1}H NMR (300 MHz, DMSO-d_6+ CCl_4): $\delta = 3.61$ (dd, $J = 18.8$, 9.9 Hz, 1H), 3.75 (s, 2H), 3.99 (dd, $J = 18.8$, 2.6 Hz, 1H), 4.44 (dd, $J = 9.9$, 2.6 Hz, 1H), 4.88 (s, 2H), 6.34 (br s, 1H), 6.44 (br s, 1H), 7.45 (br s, 1H), 7.50 (d, $J = 8.4$ Hz, 2H), 7.98 (d, $J = 8.4$ Hz, 2H), 11.75 (s, 1H); EI-MS (m/z): 462 (M^+); Anal. Calcd. for $C_{19}H_{15}ClN_4O_4S_2$: C, 49.30; H, 3.27; N, 12.10%. Found: C, 49.45; H, 3.61; N, 11.95%.

5d: Pink crystals; yield 1.44 g (63%); mp >240°C; ^{1}H NMR (300 MHz, DMSO-d_6+ CCl_4): $\delta = 2.32$ (s, 6H), 3.55 (dd, $J = 18.7$, 10.3 Hz, 1H), 3.75 (s, 2H), 3.93 (dd, $J = 18.7$, 2.4 Hz, 1H), 4.42 (dd, $J = 10.3$, 2.4 Hz, 1H), 4.87 (s, 2H), 6.32 (br s, 1H), 6.44 (br s, 1H), 7.24 (d, $J = 7.4$ Hz, 1H), 7.45 (br s, 1H), 7.70 (d, $J = 7.7$ Hz, 1H), 7.73 (s, 1H), 11.74 (s, 1H); Anal. Calcd. For $C_{21}H_{20}N_4O_4S_2$: C, 55.25; H, 4.42; N, 12.27%. Found: C, 55.11; H, 4.57; N, 12.39%.

7a: White crystals; yield 1.31 g (66%); mp 236–238°C; ^{1}H NMR (300 MHz, DMSO-d_6+ CCl_4): $\delta = 2.85$ (dd, $J = 17.4$, 10.6 Hz, 1H), 3.18 (dd, $J = 17.4$, 4.4 Hz, 1H), 4.27 (dd, $J = 10.6$, 4.4 Hz, 1H), 4.31 (s, 2H), 7.28 (d, $J = 8.8$ Hz, 2H), 7.57 (d, $J = 8.8$ Hz, 2H), 10.21 (s, 1H), 11.50 (s, 1H), 11.74 (s, 1H); Anal. Calcd. for $C_{14}H_{12}ClN_5O_3S_2$: C, 42.26; H, 3.04; N, 17.60%. Found: C, 42.12; H, 2.91; N, 17.71%.

7b: White crystals; yield 1.57 g (72%); mp 210–212°C; ^{1}H NMR (300 MHz, DMSO-d_6+ CCl_4): $\delta = 1.33$ (t, $J = 7.1$ Hz, 3H), 2.86 (dd, $J = 17.6$, 10.8 Hz, 1H), 3.17 (dd, $J = 17.6$, 4.3 Hz, 1H), 4.32 (s, 2H), 4.25 (q, $J =$ 7.0 Hz, 2H), 4.28 (dd, $J = 10.8$, 4.3 Hz, 1H), 7.67 (d, $J = 8.8$ Hz, 2H), 7.88 (d, $J = 8.8$ Hz, 2H), 10.44 (s, 1H), 11.51 (s, 1H), 11.75 (s, 1H); Anal. Calcd. for $C_{17}H_{17}N_5O_5S_5$: C, 46.89; H, 3.93; N, 16.08%. Found: C, 47.01; H, 3.82; N, 16.19%.

7c: White crystals; yield 1.80 g (69%); mp >250°C; ^{1}H NMR (300 MHz, DMSO-d_6+ CCl_4): $\delta = 2.86$ (m, 1H), 3.28 (m, 1H), 4.18 (s, 1H), 4.28 (s, 1H), 4.33 (m, 1H), 4.87 (s, 1H), 6.32 (br s, 1H), 6.39 (d, $J =$ 13.8 Hz, 1H), 7.36 (d, $J = 8.0$ Hz, 2H), 7.43 (br s, 1H), 7.51 (d, $J = 8.0$ Hz, 2H), 10.12 (d, $J = 10.7$ Hz 1H), 11.32 (s, 1H), 11.52 (s, 1H); EI-MS (m/z): 522 (M^+); Anal. Calcd. for $C_{19}H_{16}BrN_5O_4S_2$: C, 43.69; H, 3.09; N, 13.41%. Found: C, 43.82; H, 3.24; N, 13.27%.

7d: White crystals; yield 1.34 g (56%); mp >250°C; ^{1}H NMR (300 MHz, DMSO-d_6+ CCl_4): $\delta = 2.85$ (m, 1H), 3.27 (m, 1H), 4.17 (s, 1H), 4.29 (s, 1H), 4.35 (m, 1H), 4.87 (s, 1H), 6.31 (br s, 1H), 6.42 (d, $J =$ 14.1 Hz, 1H), 7.22 (d, $J = 8.4$ Hz, 2H), 7.42 (br s, 1H), 7.56 (d, $J =$

8.4 Hz, 2H), 10.13 (d, $J = 12.8$ Hz, 1H), 11.73 (s, 1H); Anal. Calcd. for $C_{19}H_{16}ClN_5O_4S_2$: C, 47.75; H, 3.37; N, 14.65%. Found: C, 47.88; H, 3.29; N, 14.52%.

7e: White crystals; yield 1.60 g (62%); mp 222–223°C; ^{1}H NMR (300 MHz, DMSO-d_6+ CCl_4): $\delta = 1.35$ (t, $J = 7.4$ Hz, 3H), 2.86 (m, 1H), 3.25 (m, 1H), 4.18 (s, 1H), 4.25(s, 1H), 4.29 (q, $J = 7.2$ Hz, 2H), 4.38 (m, 1H), 4,89 (s, 1H), 6.33 (br s, 1H), 6.40 (d, $J = 14.2$ Hz, 1H), 7.43 (br s, 1H), 7.66 (d, $J = 8.0$ Hz, 2H), 7.88 (d, $J = 8.0$ Hz, 2H), 10.31 (d, $J = 10.9$ Hz, 1H), 11.32 (s, 1H), 11.52 (s, 1H); Anal. Calcd. for $C_{22}H_{21}N_5O_6S_2$: C, 51.25; H, 4.11; N, 13.58%. Found: C, 51.39; H, 4.25; N, 13.46%.

8a: White crystals; yield 0.93 g (54%); mp >240°C; ^{1}H NMR (300 MHz, DMSO-d_6+ CCl_4): $\delta = 3.54$ (dd, $J = 18.8$, 10.2 Hz, 1H), 3.93 (dd, $J = 18.8$, 2.9 Hz, 1H), 4.28 (dd, $J = 10.2$, 2.9 Hz, 1H), 4.30 (s, 2H), 7.50 (t, $J = 7.6$ Hz, 2H), 7.61 (t, $J = 6.9$ Hz, 1H), 7.97 (d, $J = 7.5$ Hz, 2H), 11.42 (s, 1H), 11.70 (s, 1H). EI-MS (m/z): 349 (M^+). Anal. Calcd. for $C_{14}H_{12}N_4O_3S_2$: C, 48.26; H, 3.47; N, 16.08%. Found: C, 48.38; H, 3.61; N, 15.95%.

8b: White crystals; yield 1.16 g (62%); mp >240°C; ^{1}H NMR (300 MHz, DMSO-d_6+ CCl_4): δ = 2,32 (s, 6´), 3.49 (dd, $J = 18.9$, 10.5 Hz, 1H), 3.88 (dd, $J = 18.9$, 4.4 Hz, 1H), 4.26 (dd, $J = 10.5$, 4.4 Hz, 1H), 4.28 (s, 2H), 7.22 (d, $J = 7.3$ Hz, 1H), 7.67 (d, $J = 7.5$ Hz, 1H), 7.73 (s, 1H), 11.42 (s, 1H), 11.69 (s, 1H); Anal. Calcd. for $C_{16}H_{16}N_4O_3S_2$: C, 51.05; H, 4.28; N, 14.88%. Found: C, 50.99; H, 4.17; N, 14.98%.

8c: White crystals; yield 1.35 g (63%); mp 222–224°C; ^{1}H NMR (300 MHz, DMSO-d_6+ CCl_4): $\delta = 3.61$ (m, 1H), 4.05 (m, 1H), 4.23 (d, $J = 8.6$ Hz, 1H), 4.32 (s, 1H), 4.44 (m, 1H), 4.88 (s, 1H), 6.34 (br s, 1H), 6.43 (d, $J = 13.5$ Hz, 1H), 7.42 (br s, 1H), 7.51 (t, $J = 6.7$ Hz, 2H), 7.62 (t, $J = 6.7$ Hz, 1H), 7.97 (d, $J = 7.2$ Hz, 2H), 11.38 (s, 1H), 11.56 (s, 1H); Anal. Calcd. For $C_{19}H_{16}N_4O_4S_2$: C, 53.26; H, 3.76; N, 13.08%. Found: C, 53.38; H, 3.64; N, 13.15%.

8d: White crystals; yield 1.20 g (52%); mp 217–218°C; ^{1}H NMR (300 MHz, DMSO-d_6+ CCl_4): $\delta = 3.59$ (m, 1H), 3.99 (m, 1H), 4.19 (d, $J = 8.4$ Hz, 1H), 4.31 (s, 1H), 4.43 (m, 1H), 4.89 (s, 1H), 6.34 (br s, 1H), 6.39 (d, $J = 13.7$ Hz, 1H), 7.44 (br s, 1H), 7.50 (d, $J = 8.4$ Hz, 2H), 7.98 (d, $J = 8.4$ Hz, 2H), 11.37 (s, 1H), 11.75 (s, 1H); EI-MS (m/z): 462 (M^+); Anal. Calcd. for $C_{19}H_{15}ClN_4O_4S_2$: C, 49.30; H, 3.27; N, 12.10%. Found: C, 49.18; H, 3.19; N, 12.22%.

REFERENCES

[1] R. B. Lesyk and B. S. Zimenkovsky, *Curr. Org. Chem.*, **8,** 1547 (2004).

[2] M. M. Sim, S. B. Ng, A. D. Buss, S. C. Crasta, K. L. Goh, and S. K. Lee, *Bioorg. Med. Chem. Lett*., **12,** 697 (2002).
[3] B. B. Zhang and D. E. Muller, *Curr. Opin. Chem. Biol.*, **4,** 461 (2000).
[4] H. Kurogi, *Drug. Des. Discov*., **16,** 109 (1999).
[5] C. J. Bailey, *Trends Pharm. Sci.*, **21,** 259 (2000).
[6] G. J. Murphy and J. C. Holder, *Trends Pharm. Sci.*, **21,** 469 (2000).
[7] A. Degterev, A. Lugovskoy, M. Cardone, B. Mulley, G. Wagner, T. Mitchison, and J. Yuan, *Nature Cell Biol*., **3,** 173 (2001).
[8] 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, and C. P. Decicco, *Proc. Nat. Acad. Sci. U. S. A.*, **98,** 11879 (2001).
[9] H. Chen, Y.-H. Fan, A. Natarajan, Y. Guo, J. Iyasere, F. Harbinski, L. Luus, W. Christ, H. Aktas, and J. A. Halperin, *Bioorg. Med. Chem. Lett*., **14,** 5401 (2004).
[10] M. M. Turkevich and E. V. Vladsimirskaya, *Zh. Obshch. Khim*., **24**, 2010 (1954); *Chem. Abstr.,* **49,** 14737i (1955).
[11] R. Lesyk, B. Zimenkovsky, I. Subtelna, I. Nektegayev, and G. Kazmirchuk, *Acta Poloniae Pharmaceutica—Drug Research*, **6,** 457 (2003).
[12] T. Takahashi, *Tetrahedron Lett.*, **11,** 565 (1964).
[13] Developmental Therapeutics Program NCI/NIH. Available at: http://dtp.nci.nih.gov.
[14] A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, and A. Vaigro-Wolff, *J. Nat. Cancer Inst.*, **83,** 757 (1991).
[15] M. R. Boyd and K. D. Paull, *Drug Dev. Res.*, **34,** 91 (1995).
[16] M. R. Boyd, *Cancer Drug Discovery and Development* (Humana Press, Totowa, NJ, 1997), Chap. 2, pp. 23–43.
[17] C. E. Redemann, R. N. Icke, and G. A. Alles, *Org. Synth.*, **3,** 763 (1955).
[18] I. D. Komaritsa, S. N. Baranov, and A. P. Grishchuk, *Khim. Geterotsikl. Soedin.*, **4**, 664 (1967); *Chem. Abstr.*, **68,** 49495 (1968).
[19] A. P. Grischuk, I. D. Komaritsa, and S. N. Baranov, *Chem. Heterocycl. Compd.*, **2,** 541 (1966).