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Synthesis of some new bis-thiazoles as possible anticancer agents

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Several new 5-(2,3-dihydrothiazol-2-yledinyl)rhodanines **3a–c** and 5-(4-oxothiazolidinon-2-ylidenyl)rhodanine **4** were synthesized through the reaction of 5-thiocarbamoyl rhodanines **2** with phenacyl bromides or chloroacetic acid, respectively. The synthesis of the arylidene derivatives **5a–c** were also described. The 5-(4-amino-5-cyano-2,3-dihydrothiazol-2-yledinyl)rhodanines **10a, b** were obtained through reaction of rhodanines **1a, b** with thiazolium salt **9**. All the prepared compounds were screened for their anticancer activity using the NCI *in vitro* anticancer screening program. Three compounds showed promising anticancer activity against particular human cell lines used in the assay.

1. Introduction

Considerable attention has been given to synthesize compounds having two thiazole moities linked to each other either directly [1], or through different bridges, such as a sulfur bridge [2], a hydrazino bridge [3–5], a methylene bridge [6], an amino bridge [7] or others [8–12]. Most bis-thiazoles have been reported to posses various biological activities. Some of these compounds were found to have antirheumatic activity [5], others were enzyme inhibitors [9]. However, the majority of bis-thiazoles display antimicrobial [2, 6, 7, 8], antiviral [10] or antitumour [3, 4, 11, 12] activity. Many of these compounds were points of interest for major pharmaceutical companies [2–5, 10]. In a previous publication [13], we reported on the synthesis and antimicrobial activities of some new compounds having two thiazoles linked by a hydrazinocarbonyl bridge. As a continuation of our study in this direction, in the present investigation, we describe another two methods for the synthesis of new bis-thiazoles. One of the two thiazoles involved is rhodanine, a nucleus well known for its biological properties.

2. Investigations, results and discussion

2.1. Synthesis and characterization

The first method for the synthesis of bis-thiazoles involves the reaction of N-phenyl rhodanine (**1a**) with phenyl isothiocyanate in highly alkaline medium. The activated methylene group at C-5 of rhodanine attacks the isothiocyanate in a way similar to amines resulting in the formation of 5-(*N*-phenylthiocarbamoyl) rhodanine (**2**) which reacted with different phenacyl bromides to yield 5-(3,5-disubstituted-thiazolin-2-ylidenyl) rhodanines (**3a–c**). When the same intermediate **2** reacted with chloracetic acid in the presence of sodium acetate, 5-(3-substituted-4-oxothiazolidin-2-ylidenyl)rhodanine (**4**) was obtained. This product, upon condensing with different aldehydes, yielded the arylidene derivatives **7a–c**. The experiment to react the intermediate **2** with chloroacetonitril to obtain 5-(4-aminothizolidin-2-ylidenyl)rhodanine (**6**) has failed. Instead, compound **4** was obtained. A possible explanation for the formation of the 4-oxothiazolidinone instead of the 4-aminothiazolinone is that once the 4-amino compound **6** is formed, it undergoes toutomerism to the 4-imino form **7** that is converted to the 5-oxo compound by the action of HCl liberated during the reaction.

The second method involves the treatment of 4-amino-5-cyano-3-phenyl-2,3-dihydrothiazole-2(3*H*)-thione (**8**) [14] with dimethylsulfate. The produced methyl thiothiazolium salt **9** was then reacted with the selected rhodanines **1a, b** in the presence of triethylamine to form the desired bis-thiazoles **10a, b** (Scheme).

In a previous publication [15], we have studied the utilization of an exocylic active methylene group in these reactions to synthesize benzimidazolyl-thiazoles. We now have extended the application of these methods to include an active methylene group of a heterocyclic ring system. We must also point out that Gewald [16, 17] was the first who reported the use of active methylene groups of small molecules such malononitrile and ethyl cyanoacetate in similar reactions.

The spectra of the prepared bis-thiazoles are characterized by the presence of the elements identifying both thiazole rings. Compounds **3a–c** show a strong C=O absorption band at 1685–1682 cm^{-1} in the IR spectra characterizing the rhodanine nucleus and a singlet at 6.6 ppm of the thiazoline-C-4 proton in the ^1H NMR spectra. Compound **4** shows two strong C=O bands at 1735 and 1679 cm^{-1} corresponding to 4-thiazolidinone and rhodanine carbonyl absorption bands, respectively. In the arylidene derivatives **5a–c** there are no 4-thiazolidinone C-4 methylene protons of compound **4**, instead a singlet of an arylidene-H at 7.8 ppm appears. Finally, compounds **10a, b** showed the C=O absorption band of rhodanine at 1650 cm^{-1} in addition to the CN absorption band of the 4-amino-5-cyanothiazoline at 2196 cm^{-1}.

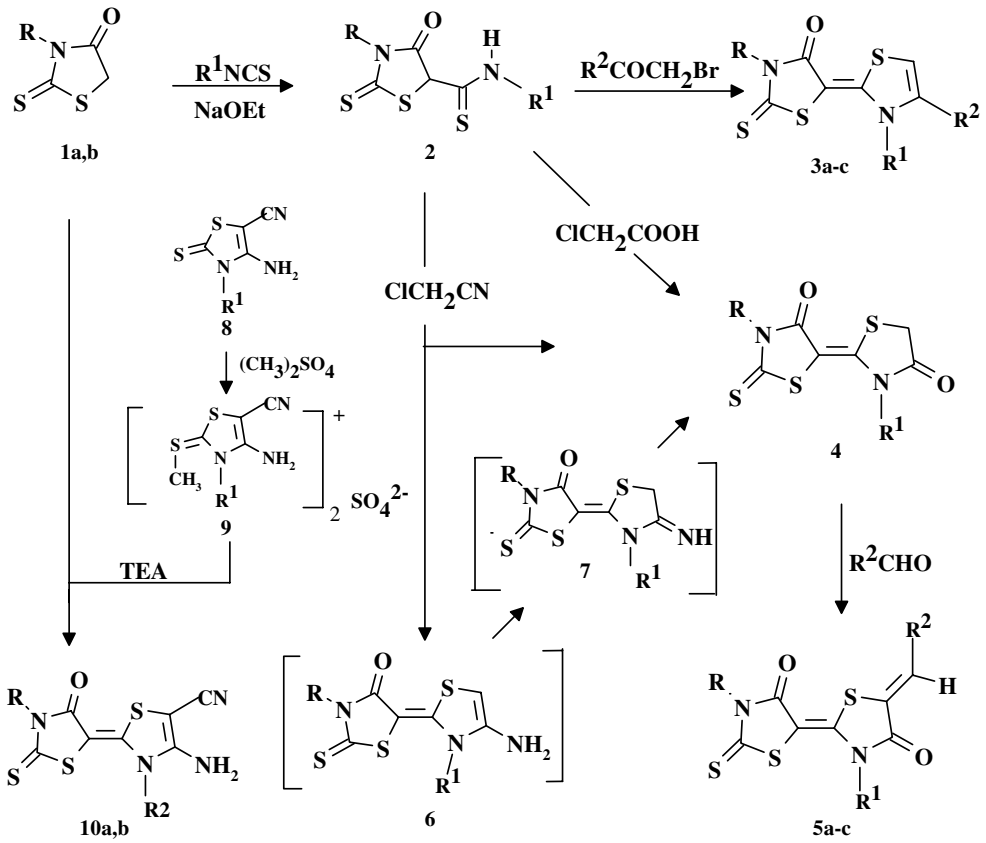
2.2. Biological results

The prepared compounds were evaluated for their antitumor activity following the NCI screening program [18–22]. They were first evaluated preliminary using the 3-cell line-one dose assay. The results of this assay are reported in Table 1. Compounds **4**, **5a**, and **5c** were considered active.

When these three compounds were passed on for the evaluation against a full panel of 60 human cell lines, they showed variable antitumor activities against most of the tested sub-panel tumor cell lines at the GI50 level. The sub-panel tumor cell lines median growth inhibitory concentration (the average sensitivity of each sub-panel towards each of the test compounds) and the full panel mean graph mid point (MG-MID) (the average of all cell lines towards each of the test compounds) are reported in Table 2.

Compound **4** is highly active against breast cancer BT-549 with a GI50 value of 2.82 μg/ml. Compound **5a** is highly active against the renal cancer CAKI-1 with a GI50 value of just 0.13 μg/ml. Compound **5c** shows high activity

Scheme



1 : R = a : C_6H_5, b: Benzyl

2,4 : R, R^1 = C_6H_5

3 : R^1=C_6H_5, R^2= a : C_6H_5, b: 4-Cl-C_6H_4, c: 4-CH_3-C_6H_4

5 : R^1=C_6H_5, R^2= a : C_6H_5, b: 4-Cl-C_6H_4, c: 4-OCH_3-C_6H_4

8,9 : R^1 = C_6H_5

10 : R = a : C_6H_5, b : Benzyl

Table 1: The 3-cell line, one-dose primary anticancer assay results

Compd.	Concentration	Growth percentage			Activity
		Lung NCI-H460	Breast MCF7	CNS SF-268	
3a	0.0001 M	93	79	69	Inactive
3b	0.0001 M	89	67	94	Inactive
3c	0.0001 M	108	89	97	Inactive
4	0.0001 M	20	19	−9	Active
5a	0.0001 M	8	19	12	Active
5b	0.0001 M	84	67	52	Inactive
5c	0.0001 M	71	67	22	Active
10a	0.0001 M	45	40	54	Inactive

against three cell lines; CNS cancer SF-268, and SNB-75 and breast cancer MCF7 with values of GI50 of 0.89, 4.61 and 0.26 μg/ml, respectively. These results are reported in Table 3. Compound **5c** retains the anticancer activity through all levels (GI50, TGI and LC50) against leukemia SR (15.3, 31.8, 66.1 μg/ml), CNS cancer SNB-75 (4.61, 21.4, 61.8 μg/ml), and renal cancer ACHN (13.6, 28.8, 60.8 μg/ml).

Table 2: Sub-panel tumor cell lines median growth inhibitory concentration (GI50) and full panel mean graph mid point (MG-MID)

Sub-panel tumor cell line	**5a**	**4**	**5c**
Leukemia	48.82	36.28	61.52
Lung cancer	38.86	35.13	60.37
Colon cancer	79.70	43.33	>100
CNS cancer	30.44	30.48	81.41
Melanoma	44.50	47.31	93.50
Ovarian cancer	47.68	38.93	71.97
Renal cancer	29.41	32.03	45.91
Prostate cancer	46.05	31.30	>100
Breast cancer	55.72	34.24	67.22
MG-MID	33.88	38.01	45.70

MG-MID: average sensitivity of each sub-panel and of all cell lines towards each of the test compounds

The ratio obtained by dividing the compound's full panel MG-MID (μM) by its individual sub-panel MG-MID (μM) is considered as a measure of compound selectivity. Ratios between 3 and 6 refer to moderate selectivity, ratios > 6 indicate high selectivity towards the corresponding cell line, while compounds not meeting either of these

Table 3: The most affected cell lines and the corresponding GI50 and sensitivity towards each of the active compounds

Compd.	Most affected cell line	GI50	MG-MID	Sensitivity ratio
4	Breast BT-549	2.82 μm/ml	33.88	12
5a	Renal CAKI-1	0.13 μm/ml	38.01	292
5c	CNS SF-268	0.89 μm/ml	45.70	51
	CNS SNB-75	4.61 μm/ml		10
	Breast MCF7	0.26 μm/ml		176

criteria are rated non-selective [21]. All the active compounds in the present study prove to be non-selective against any of the nine tumor sub-panels tested. However, regarding the selectivity of the tested compounds against the highly affected individual tumor cell lines, compound **4** exhibits high selectivity towards breast cancer BT-549 at the GI_{50} level with a selectivity ratio 12. On the other hand, compound **5a** shows great selectivity against renal cancer CAKI-1 sub-panel tumor cell line at the GI_{50} level with a selectivity ratio of 292. Compound **5c** is very selective against the CNS cancer SF-268, SNB-75 and Breast cancer MCF7 with selectivity ratios of 51, 10 and 176 respectively (Fig.).

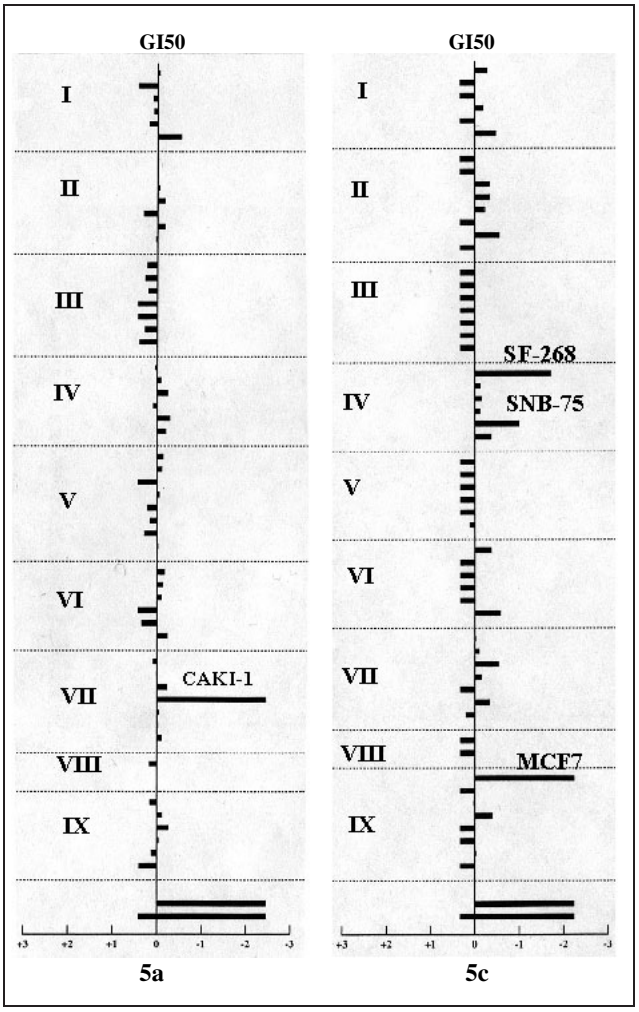


Fig.: GI50-based mean graphs [22] from screening of compound **5a** and **5c** in the NCI human tumor cell line panel that are identified as follows: I(leukemia) II(lung, non-small cell), III(colon), IV(CNS), V(melanoma), VI(ovarian), VII (renal), VIII(prostate), IX(breast). Bars extending to the right represent sensitivity of cell line to the test agents in excess of the average sensitivity of all tested cell lines. Since the bar scale is logarithmic, a bar extending unit 2 units to the right implies that the compound achieve GI50 for that cell line at a concentration one hundredth the mean concentration required over all cell lines

The activity of the tested compounds could be tentatively correlated to the structure variations and modifications. Only one group of compounds, the 4-oxothiazolidin-2-ylidenyl-4-oxothiazolidin-2-thione **4** and its arylidene derivatives **5a, c** exhibit antitumor activities. Among the arylidene derivatives, only the unsubstituted and the 4-methoxy derivatives are active while the 4-chloro analogue is inactive. Finally, the high degree of cell-sensitivity and selectivity expressed by compounds **5a** and **5c** against renal cancer CAKI-1 and breast cancer MCF7 (selectivity ratios of 292 and 175, respectively) raises the potentiality of their future derivatization in order to explore scope and limitations of their activities.

3. Experimental

3.1. Apparatus

Melting points were determined in open glass capillaries on Griffen apparatus and are uncorrected. IR spectra (KBr) were recorded on a Perkin-Elmer 1430 spectrometer. ^{1}H-NMR spectra were determined on a Varian 300 MHz device, using TMS as an internal standard; chemical shifts are given in δ (ppm). Microanalyses were carried out using a Perkin-Elmer RE 2400 C H N S Analizer. All values of C, H, N and S are within ± 0.4% of the calculated data. Product characteristics are given in Table 4.

3.2. Synthesis of the compounds

3.2.1. 5-(N-Phenylthiocarbamoyl)-3-phenyl-4-oxothiazolidin-2-thione (**2**)

To a stirred solution of sodium ethoxide (0.23 g sodium in 7.5 ml of absol. ethanol), 3-phenyl-4-oxothiazolidin-2-thione (**1a**) (2.02 g, 10 mmol) and phenyl isothiocyanate (1.35 m, 1.19 ml, 10 mmol) were added. The reaction mixture was stirred at room temperature for 30 min then it was neutralized with glacial acetic acid. The product obtained was filtered, washed with ethanol, dried and recrystallized from aqueous dimethylformamide. IR (KBr, cm^{-1}): 1693 (C=O), 1595, 1494 (C=C), 1555, 1234, 1184 (N−C=S).

3.2.2. 5-(4-Substituted-pheny-3-phenyl-2,3-dihydrothiazol-2-ylidenyl)-3-phenyl-4-oxothiazolidin-2-thiones (**3a–c**)

To a solution of **2** (3.4 g, 10 mmol) in glacial acetic acid (20 ml), anhydrous sodium acetate (0.82 g, 10 mmol) and substituted phenacylbromide (10 mmol) were added. The reaction mixture was heated under reflux for 3 h. After cooling, the product was filtered, washed with ethanol, dried and recrystallized from glacial acetic acid.

Table 4: Melting points, yields, molecular formulae and molecular weights of the newly prepared bis-thiazoles

Compd.	R	R^1	R^2	M.p. (°C)	Yield (%)	Mol. formula (Mol. Wt.)
2	C_6H_5	C_6H_5	–	122-2	75	$C_{16}H_{12}N_2OS_3$ (344.48)
3a	C_6H_5	C_6H_5	C_6H_5	>300	70	$C_{24}H_{16}N_2OS_3$ (444.60)
3b	C_6H_5	C_6H_5	4-ClC_6H_4	>300	71	$C_{24}H_{15}ClN_2OS_3$ (479.05)
3c	C_6H_5	C_6H_5	4-$CH_3C_6H_4$	>300	72	$C_{25}H_{18}N_2OS_3$ (458.63)
4	C_6H_5	C_6H_5	–	>300	75	$C_{18}H_{12}N_2O_2S_3$ (384.50)
5a	C_6H_5	C_6H_5	C_6H_5	>300	75	$C_{25}H_{16}N_2O_2S_3$ (472.61)
5b	C_6H_5	C_6H_5	4-ClC_6H_4	>300	77	$C_{25}H_{15}ClN_2O_2S_3$ (507.06)
5c	C_6H_5	C_6H_5	4-$OCH_3C_6H_4$	>300	76	$C_{26}H_{18}N_2O_3S_3$ (502.64)
10a	C_6H_5	C_6H_5	C_6H_5	>300	74	$C_{19}H_{12}N_4OS_3$ (408.53)
10b	C_6H_5	C_6H_5	$CH_2H_6H_5$	>300	73	$C_{20}H_{14}N_4OS_3$ (422.55)

3a: IR (KBr, cm^{-1}) 1682 (rhodanine-C=O), 1593, 1480 (C=C), 1239,1098 (N−C=S). ^{1}H NMR (*DMSO*-d_6): δ (ppm) 6.6 (s, 1 H, thiazoline-4-H), 7.1–7.5 (m, 15H, Ar-H).
3b: IR (KBr, cm^{-1}) 1685 (rhodanine-C=O), 1595, 1485 (C=C), 1240, 1100 (N−C=S). ^{1}H NMR (*DMSO*-d_6): δ (ppm) 6.6 (s, 1 H, thiazoline-4-H), 7.2–7.6 (m, 14H, Ar−H).
3c: IR (KBr, cm^{-1}): 1685 (rhodanine-C=O), 1593, 1489 (C=C), 1244, 1095 (N−C=S). ^{1}H NMR (*DMSO*-d_6): δ (ppm) 2.3 (s, 3H, tolyl-CH_3), 6.6 (s, 1 H, thiazoline-4-H), 7.1–7.5 (m, 15 H, Ar−H).

3.2.3. 5-(3-Phenyl-4-oxothiazolidin-2-ylidenyl)-3-phenyl-4-oxothiazolidin-2-thione (**4**)

To a suspension of **2** (3.4 g, 10 mmol) in glacial acetic acid (20 ml), anhydrous sodium acetate (0.83 g, 10 mmol) and chloroacetic acid (0.94 g, 10 mmol) were added. The reaction mixture was heated under reflux for 2 h, during which the product crystallized as shiny yellow crystals. After cooling, the product was filtered, washed with EtOH, dried and recrystallized from glacial acetic acid.
IR (KBr, cm^{-1}): 1735 (C=O), 1679 (C=O), 1595, 1494 (C=C), 1546, 1238, 1170
(N−C=S). ^{1}H NMR (*DMSO*-d_6): δ (ppm) 4.0 (s, 2 H, CH_2), 7.2–7.7 (m, 10 H, Ar−H).

3.2.4. 5-(4-Substituted-benzylidene-3-phenyl-4-oxothiazolidin-2-ylidenyl)-3-phenyl-4-oxothiazolidin-2-thiones (**5a–c**)

To a solution of **4** (3.8 g, 10 mmol) and piperidine (1 ml) in absol. ethanol (20 ml), the pertinent aldehyde (10 mmol) was added. The reaction mixture was heated under reflux for 3 h during which the product crystallized as shiny yellow crystals. After cooling, the product was filtered, washed with EtOH, dried and recrystallized from glacial acetic acid.
5a: IR (KBr, cm^{-1}): 1710 (thiazolidinone-C=O), 1684 (rhodanine-C=O), 1590, 1495 (C=C), 1550, 1240, 1150 (N−C=S). ^{1}H NMR (*DMSO*-d_6): δ (ppm) 7.2–7.6 (m, 15 H, Ar−H), 7.8 (s, 1 H, =C−H).
5b: IR (KBr, cm^{-1}): 1709 (thiazolidinone-C=O), 1686 (rhodanine-C=O), 1595, 1495 (C=C), 1553, 1240, 1150 (N−C=S). ^{1}H NMR (*DMSO*-d_6): (δ ppm) 7.2–7.6 (m, 14 H, Ar−H), 7.8 (s, 1 H, =C-H).
5c: IR (KBr, cm^{-1}): 1709 (thiazolidinone-C=O), 1686 (rhodanine-C=O), 1589, 1514 (C=C), 1564, 1240, 1152 (N-C=S). ^{1}H NMR (*DMSO*-d_6): (δ ppm) 3.9 (s, 3 H, OCH_3), 7.2–7.7 (m, 14 H, Ar−H), 7.8 (s, 1 H, =C−H).

3.2.5. 5-(4-Amino-5-cyano-3-phenyl-2,3-dihydrothiazol-2-ylidenyl)-3-phenyl-4-oxothiazolidin-2-thiones (**10a**, **b**)

To a solution of 4-amino-5-cyano-3-phenyl-2,3-dihydrothiazol-2-thione (**8**) [14] (2.33 g, 10 mmol) in acetonitrile (20 ml), dimethylsulfate (1.9 g, 1.45 ml, 15 mmol) was added. The reaction mixture was heated under reflux for 30 min and then cooled. 3-Substituted-4-oxothiazolidin-2-thiones (**1a, b**) (10 mmol) and triethyl amine (1 ml) were added while stirring. Stirring was continued for 10 min in a boiling water bath. Shiny orange crystals separated. After cooling, the product was filtered, washed with EtOH, dried and recrystallized from aqueous DMF.
10a: IR (KBr, cm^{-1}): 3457, 3310 (NH_2), 2196 (C=N), 1650 (rhodanine-C=O), 1586, 1515 (C=C), 1552,1197, 1037, 961 (N−C=S).
10b: IR (KBr, cm^{-1}): 3457, 3310 (NH_2), 2196 (C=N), 1650 (rhodanine-C=O), 1586, 1515 (C=C), 1552,1197, 1037, 961 (N−C=S). ^{1}H NMR (*DMSO*-d_6): (δ ppm) 4.0 (s, 2H, Bn-CH_2), 7.2–7.65 (m, 10 H, Ar−H).

3.3. Anticancer screening

The prepared compounds were evaluated for their anticancer activities using the NCI *in vitro* anticancer screening assay [18–22].

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