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Arylsulfonyl-*N*,*N*-diethyl-dithiocarbamates: A Novel Class of Antitumor Agents

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Abstract—A series of alkyl/arylsulfonyl-*N*,*N*-diethyl-dithiocarbamates has been prepared by reaction of sodium *N*,*N*-diethyl-dithiocarbamate with alkyl/arylsulfonyl halides. The reactivity of these new derivatives against cysteine and glutathione has been investigated in order to identify derivatives that might label a critical cysteine residue of tubulin (Cys 239 of human β2 tubulin chain). Some of the most reactive compounds showed moderate to powerful tumor growth inhibitory properties against several leukemia, non-small cell lung, ovarian, melanoma, colon, CNS, renal, prostate and breast cancer cell lines in vitro.

Introduction

A common feature of several types of antitumor agents recently reported consists of the presence of primary/secondary sulfonamide moieties in their molecules.[1–5] Thus, some arylsulfonyl-ureas/hydroxyguanidines[1,2] or sulfonimideamides[3] of types **1–3** have been reported by researchers from Eli Lilly and by Chern et al.,[4] whereas Medina's group[5–7] prepared *N*-substituted polyhalogenobenzenesulfonamides all of which include type **4** compounds, which strongly inhibited the growth of multidrug resistant MCF-7/ADR cancer cells in vitro. Other antitumor sulfonamides that have been investigated include CQS, 5-chloroquinoxaline-2-sulfanilamide **5**,[8,9] E7010 **6**,[10] and E7070 **7**.[11] Furthermore, this group reported recently powerful tumor growth inhibition with sulfonamides incorporating alkyldithiocarbamyl moieties of types **8** and **9**.[12,13] The mechanisms of antitumor action of many of these compounds are still unclear,[14] but at least E7010 **6** and the perfluoroarylsulfonyl derivatives **4** were shown to act as tubulin polymerization inhibitors, binding at the colchicine site, and modifying Cys 239 of this protein, respectively.[5–7,10] On the other hand, the molecular targets of sulfonamides **8**, **9** and E7070 might be some carbonic anhydrase isozymes predominantly present in tumor cells.[12,13,15]

Some of the most widely used antitumor drugs (such as the vinca alkaloids among others)[16] exert their action by binding to tubulin, a heterodimeric (αβ) protein that is the key component of microtubules and thus of the cytoskeleton, and which plays a crucial function in cell division.[7,17–20] The same mechanism of action has also been shown by some of the compounds **1–9** mentioned above. Among them, the most interesting seems to be the pentafluorophenylsulfonamides **4** which strongly inhibit tumor growth due to their nucleophilic aromatic substitution reaction with Cys 239 of the β chain of some tubulin isoforms, resulting thus in the disruption of cellular microtubules followed by apoptosis.[5–7] Since some compounds containing dialkyldithiocarbamylsulfenyl moieties (incorporated in the antitumor sulfonamides **8** and **9** for example) might also react with the SH group of cysteine or cysteine-containing peptides/proteins, it appeared of interest to investigate whether such derivatives may lead to effective antitumor agents. Here we report a new class of such derivatives, i.e., the arylsulfonyl-*N*,*N*-diethyl-dithiocarbamates, obtained by reaction of sodium *N*,*N*-diethyldithiocarbamate with sulfonyl halides. Some of the new derivatives reported here showed promising in vitro tumor growth inhibitory properties against a multitude of leukemia, non-small cell lung, ovarian, melanoma, colon, CNS, renal, prostate and breast cancer cell lines.

Reaction of alkyl/arylsulfonyl halides with sodium *N*,*N*-diethyldithiocarbamate (in acetone–water) afforded a series of alkyl/arylsulfonyl-*N*,*N*-diethyl-dithiocarbamates

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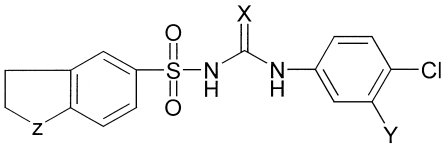
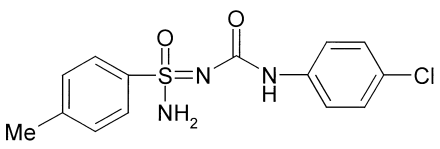


1a: X = O, Y = H, Z = CH_2 - sulofenur
1b: X = O, Y = Cl, Z = O - LY295501
1c: X = S, Y = H, Z = CH_2



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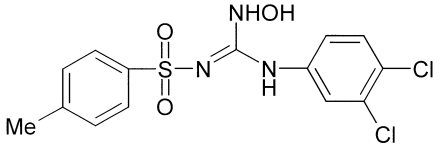

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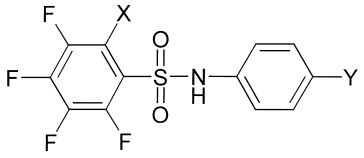
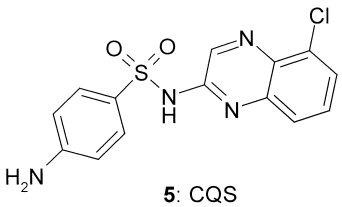
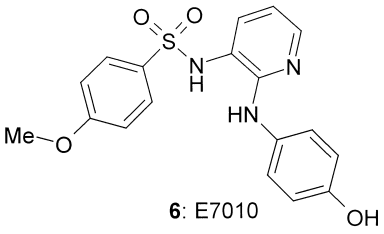


4: X = H, F, Br
Y = OMe; NH_2; NMe_2



5: CQS



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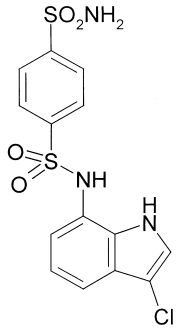
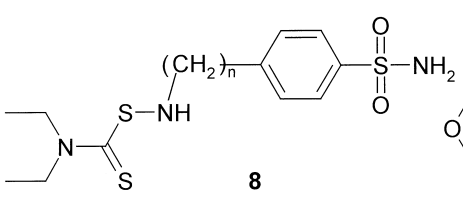


7: E7070



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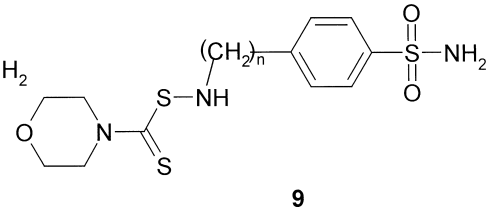


9

n = 0
n = 1
n = 2

K_I = 10 - 35 nM (hCA II)
GI_{50} = 10 - 65 nM
(different tumor cell lines)

10a–z (Table 1). All these compounds were characterized by spectral and elemental analysis data that confirmed their structure. The reaction of these new derivatives with cysteine (Cys) and glutathione (Glt) has been investigated by means of HPLC, by incubating equimolar amounts of **10** and Cys/Glt in phosphate buffer at 37 °C for 24 h. The amount of labeled Cys/Glt is also shown in Table 1, proving that some arylsulfonyl-*N*,*N*-diethyl-dithiocarbamates indeed react with these nucleophiles under the conditions of our experiments, whereas the corresponding alkyl/perfluoroalkyl-substituted compounds do not show any reactivity. Among the best thiol group modifiers were the 2- and 4-nitro-; 2-carboxy-; 4-amino- and 4-acetamido substituted derivatives **10**. All these compounds generally reacted more rapidly with cysteine than with glutathione.

Several of the most reactive arylsulfonyl-*N*,*N*-diethyl-dithiocarbamates **10** (such as **10i**, **k**, **m**, **n**, **r**) against the thiol reagents mentioned above were then tested for their tumor growth inhibitory properties (Table 2).[21]

The following should be noted regarding the tumor cell growth inhibition data with the test compounds **10**: (i) different cancer cell lines of the same tumor type possessed a variable response to inhibition of growth in the presence of the new derivatives. For example, the MOLT-4 leukemia cells were very susceptible to inhibition by **10i** (GI_{50} of 0.4 μM), whereas other leukemia cell lines (such as RPMI-8226; K-562) showed the same level of inhibition only at concentrations between 11–15 μM of inhibitor. The same situation has been seen in the case of diverse non-small cell lung cancer cell lines,

Table 1. Alkyl/arylsulfonyl-*N*,*N*-diethyl-dithiocarbamates **10** prepared in the present study, and their reactivity against thiol reagents (cysteine, Cys, and glutathione, Glt). $RSO_2SC(=S)NEt_2$ **10a**–**z**[a]

Compound **10**	R	Synthesis method	% SH modification	
			Cys	Glt
a	Me_2N-	A	0	0
b	Me	A	0	0
c	Et	A	2	0
d	*n*-Pr	A	4	0
e	CF_3-	A	5	0
f	*n*-C_4F_9-	B	6	2
g	*n*-C_8F_{17}	B	4	0
h	$PhCH_2$-	B	8	0
i	*o*-O_2N-C_6H_4-	A	85	69
j	*m*-O_2N-C_6H_4-	A	36	12
k	*p*-O_2N-C_6H_4-	A	78	57
l	3-Cl-4-O_2N-C_6H_3-	A	56	39
m	*p*-AcNH-C_6H_4-	A	62	60
n	*p*-H_2N-C_6H_4-	B	58	61
p	*m*-H_2N-C_6H_4-	B	30	26
q	C_6F_5-	B	14	10
r	*o*-HOOC-C_6H_4-	C	66	35
s	*o*-HOOC-C_6Br_4-	C	29	18
t	4-CH_3O-3-H_2N-C_6H_3-	A	24	22
u	2-HO-3,5-Cl_2-C_6H_2-	A	38	36
v	4-Me_2N-C_6H_4-N=N-C_6H_4-	A	17	11
x	5-Dimethylamino-1-naphthyl-	A	15	15
y	1-Naphthyl	A	14	13
z	2-Naphthyl	A	18	16

[a]A—Sodium *N*,*N*-diethyldithiocarbamate (**11**) + RSO_2Cl; B—**11** + RSO_2F; C—**11** + sulfobenzoic cyclic anhydride.

with **10k** acting as a very potent inhibitor ($GI_{50} = 20$ nM) against the NCI-H522 line, whereas the related NCI-H226 line showed the same level of inhibition at concentrations as high as 83 μM. Other cell lines of this tumor, such as HOP-62, had an intermediate behavior between the two extremes reported above ($GI_{50} = 32$ μM); (ii) all the investigated cancer lines were generally inhibited by one or the other sulfonamides tested, but some types of tumors, such as the leukemia, non-small cell lung or ovarian ones, were generally more susceptible to inhibition, whereas others, such as the colon, renal CNS, melanoma, breast or prostate cancer cell lines were less susceptible; (iii) some of the tumors investigated here responded very well to inhibition with the new compounds **10**, with GI_{50} values in the nanomolar range. These included: SR leukemia with **10m**; NCI-H522 non-small cell lung cancer with all these derivatives; IGROV1 ovarian cancer with **10i** (Table 2). The largest majority of susceptible tumors was inhibited at micromolar concentrations of the test compounds, with GI_{50} values in the range of 1–60 μM; (iv) important differences of activity between the investigated compounds **10** were detected, with the 2-nitro and 4-acetamido derivatives showing broad tumor inhibitory properties against almost all the investigated cell lines, whereas other compounds, such as the 2-carboxy- or 4-amino-substituted ones showed a much poorer broad activity, being particularly active only against several types of tumor cell lines. A somehow intermediate behavior was shown by the 4-nitro-derivative **10k**; (v) the inhibition of growth of tumor cells was dose-dependent of the concentration of test compound used in the experiments (data not shown), with growth inhibition increasing at increasing concentrations of arylsulfonyl-*N*,*N*-diethyldithiocarbamate.

Table 2. In vitro tumor growth inhibition data with some of the new compounds **10** synthesized in the present work[21]

Tumor	Cell line	GI_{50} (μM)[a]				
		10r	**10i**	**10k**	**10n**	**10m**
Leukemia	HL-60 (TB)	1.4	0.2	—	>100	24
	MOLT-4	—[b]	0.4	—	2	48
	K-562	—	11	19	34	69
	SR	3.6	0.3	0.1	3	0.01
	CCRF-CEM	82	2.5	—	>100	20
	RPMI-8226	—	15	12	22	18
Non-small cell lung cancer	A549/ATCC	6.5	3	1	7	0.2
	HOP-62	>100	20	32	33	18
	HOP-92	>100	14	4	5	16
	NCI-H226	>100	36	83	98	35
	NCI-H522	1.2	0.08	0.02	1	<0.01
Colon cancer	COLO-205	>100	28	19	>100	22
	HCT-15	>100	34	52	>100	28
	HT29	>100	14	22	—	—
	SW-620	>100	15	18	>100	16
	HCC-2998	—	24	94	8	27
	HCT-116	—	15	5	>100	15
	KM12	—	12	11	22	13
CNS cancer	SF-268	>100	26	>100	>100	44
	SF-295	>100	32	59	>100	28
	SF-539		—	20	21	—
	SNB-75	>100	76	77	48	32
	U251	>100	13	14	>100	15
Melanoma	LOX IMVI	>100	16	17	>100	14
	M14	>100	11	15	63	15
	MALME-3M	91	10	14	46	16
	UACC-257	>100	17	24	>100	9
	SK-MEL-28	>100	13	19	>100	15
	SK-MEL-5	37	3	2	13	0.4
Ovarian cancer	IGROV1	>100	0.5	—	5	12
	OVCAR-4	>100	23	45	>100	22
	OVCAR-3	>100	22	35	>100	31
	OVCAR-8	>100	18	20	>100	15
Renal cancer	768-0	>100	19	16	3	17
	ACHN	>100	24	36	61	27
	CAKI-1	>100	17	18	22	16
	RXF 393	>100	15	16	22	11
	UO-31	>100	13	21	80	20
	TK-10	—	29	43	>100	48
	SN12C	—	18	27	>100	18
Prostate cancer	PC-3	>100	54	—	90	—
	DU-145	>100	19	27	>100	23
Breast cancer	MCF7	>100	25	6	5	18
	MDA-MB-231/ATCC	>100	16	26	>100	20
	NCI/ADR-RES	—	30	48	>100	27
	MDA-N	>100	19	30	65	21
	HS 578T	—	29	>100	83	60
	MDA-MB-435	—	18	23	>100	19
	BT-549	—	26	36	>100	61

[a]Molarity of inhibitor producing a 50% inhibition of growth of the tumor cells after 48 h exposure to variable concentrations (10^{-4}–10^{-8} M) of the test compound. Errors were in the range of ±5–10% (from two determinations).
[b]All over the table, this sign means that the compounds have not been tested for the inhibition of growth of these tumor lines.

The precise mechanism of tumor growth inhibition with these arylsulfonyl-*N*,*N*-diethyl-dithiocarbamates is not

known for the moment, but a hypothesis may be done in this regard, which has in part been verified by the experiments described below. Thus, we performed some preliminary investigations in order to show that the cellular target of these compounds is indeed tubulin. The in vitro tubulin polymerization reaction has been investigated turbidimetrically in the presence of well known tubulin polymerization inhibitors such as colchicine, or the sulfonamide **4a** (X = F; Y = OMe),[5–7] as well as one of the most active compounds described here, **10m** [5,22,23] (Fig. 1). It may be seen that both colchicine (at a concentration of 3 μM — curve 3) as well as the recently reported compound **4a**[5–7] (at the same concentration — curve 5) strongly inhibit microtubule formation. The same type of behavior has been seen with the new derivative **10m** reported in the present paper. At a concentration of 2 μM (curve 2), this compound only slightly inhibited the above mentioned reaction, but raising its concentration at 4 μM (curve 4) led to a highly increased microtubule formation. Its behavior at this concentration is intermediate between that of colchicine and the strong inhibitor **4a**.[23]

From the above data, one may conclude that similarly with sulfonamide **4a** (X = F; Y = OMe),[5–7] the compounds described here might modify the SH moiety of Cys 239 of tubulin β1, 2 or 4 chains, thus leading to disruption of cellular microtubules. A possible labeling of tubulin by one of the arylsulfonyl-*N*,*N*-diethyl-dithiocarbamates reported here is shown schematically in Scheme 1.

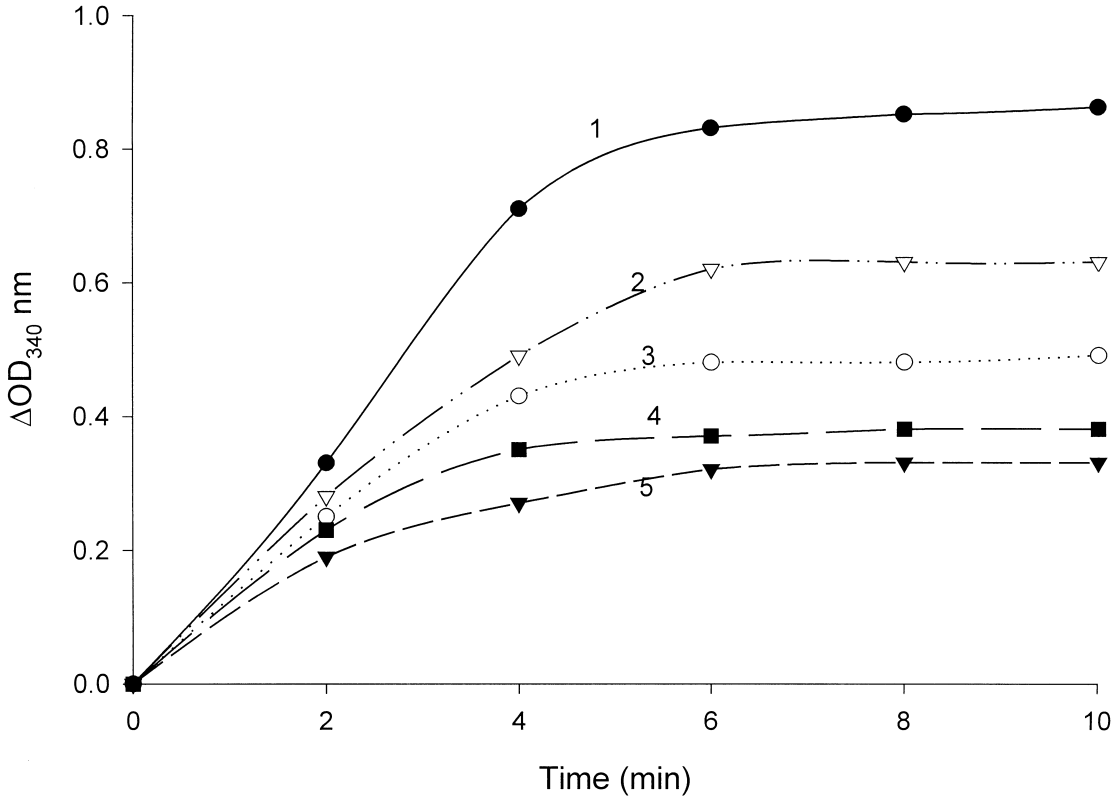
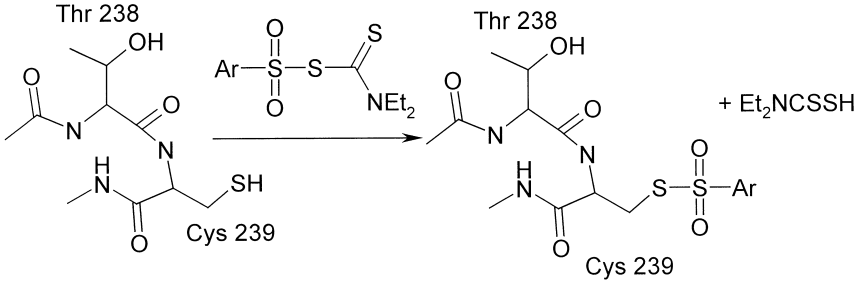


Figure 1. In vitro tubulin polymerization turbidimetric assay. Changes of OD at 340 nm over time in curve 1—with no drug added, curve 2—2 μM compound **10m**; curve 3—3 μM colchicine; curve 4—4 μM compound **10m**; curve 5—3 μM compound **4a**.



Scheme 1.

Acknowledgements

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21. Inhibition of tumor cell growth was determined at NIH, National Cancer Institute (Bethesda, MD, USA) as follows: stock solutions of inhibitor (1 mM) were prepared in DMSO, and dilutions up to 10 nM prepared with distilled deionized water. The percentage growth (PG) of the cell lines in the presence of five concentrations (10^{-8}–10^{-4} M) of inhibitor was calculated according to one of the following two expressions (1) or (2):

$$\mathrm{PG} = 100 \times (\text{Mean OD}_{\text{test}} - \text{Mean OD}_0)/(\text{Mean OD}_{\text{ctrl}} - \text{Mean OD}_0), \quad \text{when } (\text{Mean OD}_{\text{test}} - \text{Mean OD}_0) \geq 0, \tag{1}$$

$$\mathrm{PG} = 100 \times (\text{Mean OD}_{\text{test}} - \text{Mean OD}_0)/\text{Mean OD}_0, \quad \text{when } (\text{Mean OD}_{\text{test}} - \text{Mean OD}_0) < 0, \tag{2}$$

where:

Mean OD_0 = the average optical density measurements of sulforhodamine B (SRB)-derived color just before exposure of cells to the test compounds;
Mean OD_{test} = the average optical density measurements of SRB-derived color after 48 hours exposure of cells to the test compounds;
Mean OD_{ctrl} = the average optical density measurements of SRB-derived color after 48 hours with no exposure of cells to the test compounds.

GI_{50} represents the molarity of inhibitor producing a 50% inhibition of growth of the tumor cells after 48 h exposure to variable concentrations (10^{-4}–10^{-8} M) of the test compound, measured as outlined before, and this parameter was obtained by interpolation. GI_{50} is in fact the molarity of inhibitor at which PG = 50%. The standard sulforhodamine B (SRB) protein assay has been used to estimate cell viability or growth (cf. Teicher, B. A. Ed.; *Anticancer Drug Development Guide: Preclinical Screening, Clinical Trials, and Approval*; Humana Press Inc.: Totowa, NJ, 1997; pp 7–125).
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23. The in vitro tubulin polymerization reaction has been assayed as described in refs 7 and 22. Ice-cold bovine brain tubulin (from Sigma) solution (400 μg in 80 mM Pipes, pH 6.8/0.5 mM $MgCl_2$/1 mM EGTA buffer-BRB80 buffer) supplemented with 10% glycerol was mixed with 49 μL of a cold GTP solution (10 mM) and 1 μL of DMSO (or DMSO solution of the test compound). The mixture was transferred to a quartz cuvette equilibrated at 37 °C. Changes in the optical density (OD) at >340 nm were registered every 30 s, maintaining the temperature at 37 °C, with a Perkin Elmer spectrophotometer. Colchicine was from Sigma, whereas compound **4a** used as standard in the assay was synthesized from pentafluorophenylsulfonyl chloride and *p*-anisidine, as described in ref 5.