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Arylsulfonyl-N,N-dialkyl-dithiocarbamates as Tumor Cell Growth Inhibitors: Novel Agents Targeting β-Tubulin?

ANDREA SCOZZAFAVA[a], ANTONIO MASTROLORENZO[b] and CLAUDIU T. SUPURAN[a,*]

[a]*Università degli Studi, Laboratorio di Chimica Inorganica e Bioinorganica, Via Gino Capponi 7, I-50121, Florence, Italy;* [b]*Università degli Studi, Dipartimento di Scienze Dermatologiche, Centro MTS, Via degli Alfani 37, 50122 Firenze, Italy*

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Reaction of sodium N,N-dimethyldithiocarbamate or N,N-diethyldithiocarbamate with arylsulfonyl halides afforded a series of arylsulfonyl-N,N-dialkyl-dithiocarbamates. The reactivity of these new derivatives with cysteine and glutathione has been investigated in order to identify derivatives that might label a cysteine residue of the heterodimeric protein tubulin which plays a critical physiological function in cell division and also possesses enzymatic activity as a GTP-ase. Since many antitumor drugs exert their action by binding to tubulin, inhibiting in this way microtubule association and provoking cell death, some of the most reactive compounds against the thiol reagents found in this work have been assayed for their antitumor activity. Indeed strong tumor cell growth inhibitory properties against several leukemia, non-small cell lung, ovarian, melanoma, colon, CNS, renal, prostate and breast cancer has been found *in vitro* for some of the 4-halogeno-, 4-methyl- or 4-carboxyphenyl-substituted arylsulfonyl-N,N-dialkyl-dithiocarbamates. Furthermore, some of these derivative were shown to act as *in vitro* tubulin polymerization inhibitors using a turbidimetric assay.

Keywords: N,N-dialkyl-dithiocarbamates, arylsulfonyl-N,N-dialkyl-dithiocarbamates, tubulin, antitumor agent

INTRODUCTION

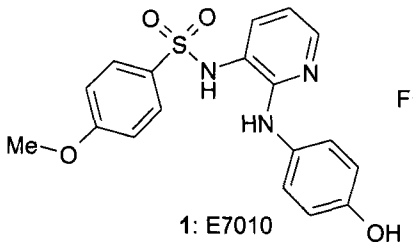
Many widely used antitumor drugs (such as the vinca alkaloids, the taxanes paclitaxel and docetaxel or the new anticancer agents combrestatin or 1-aryl-3-(2-chloroethyl)-ureas among others)[1–4] exert their action by binding to tubulin, thus blocking cells in the G2/M phase and leading to apoptosis.[5–9] Tubulin, a heterodimeric ($\alpha\beta$) protein, is the key component of microtubules and thus of the cytoskeleton, and plays a crucial function in cell division.[1–9] Tubulins also possess enzymatic activity, behaving as an effective GTP-ase.[9] At least six β-tubulin isoforms have been identified up to now that differ primarily in the carboxy-terminal 15 amino acids and possess distinct affinities for diverse chemotherapeutic agents.[4,10] Furthermore, modification of β-tubulin by mutation, or differential expression of its isozymes, seems to be responsible for either *de novo* or acquired resistance to chemotherapeutic agents.[11] It is thus essential to design novel types

*Corresponding author. Tel.: 39-055-2757551. Fax: +39-055-2757555. E-mail: cts@bio.chim.unifi.it.

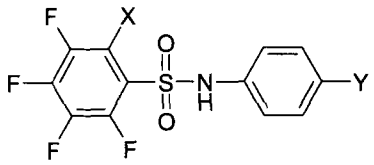
of pharmacological agents that may escape these mechanisms. Among the most interesting such compounds reported in the last period one should mention the secondary sulfonamide E7010 **1**,[12] which acts as a potent tubulin polymerization inhibitor, binding at the colchicine site of the protein,[12] or the N-substituted polyhalogeno-benzenesulfonamides of type **2** which strongly inhibit the growth of multidrug resistant MCF-7/ADR cancer cells *in vitro*, due to covalent modification of Cys 239 from the tubulin β chain as a consequence of a nucleophilic aromatic substitution reaction involving the 4-fluoro atom of the cytotoxic agent.[6–9]

In previous papers, our group reported the powerful tumor growth inhibition observed with some sulfonamides incorporating alkyldithiocarbamyl moieties of types **3** and **4**,[13,14] as well as with the structurally related arylsulfonyl-N,N-diethyl-dithiocarbamates **5**.[15]

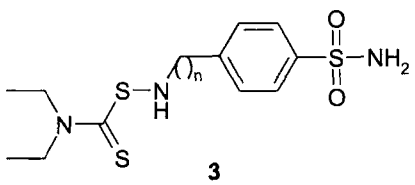
Compounds **5** have been designed by taking into consideration the fact that the dialkyldithiocarbamylsulfenyl moieties incorporated in the antitumor sulfonamides **3** and **4** might modify the SH group of cysteine or cysteine-containing peptides/proteins, constituting thus an unexplored possibility for obtaining novel types of agents that might interfere with β tubulin. Indeed, some of the derivatives **5** which were shown to readily react with cysteine or reduced glutathione, possessed strong tumor growth inhibitory properties against a wide range of cancer cell lines *in vitro*, and also inhibited the tubulin polymerization reaction *in vitro*.[15] Here we extend our previous (preliminary) study[15] on this class of antitumor agents, reporting the



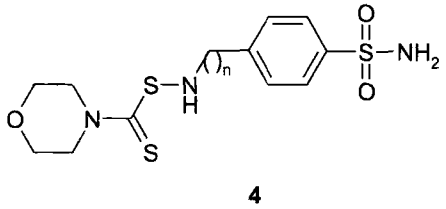
1: E7010



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



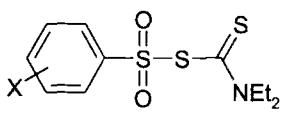
3



4

n = 0
n = 1
n = 2

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



5: X = NO_2, NH_2, NHAc

synthesis of some new arylsulfonyl-N,N-dialkyl-dithiocarbamates. Some of the derivatives reported here also 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 and inhibited the tubulin polmerization *in vitro*.

MATERIALS AND METHODS

Melting points were done on a heating plate microscope (not corrected), IR spectra as KBr pellets on a 400–4000 cm^{-1} Perkin-Elmer 16PC FTIR spectrometer and ^{1}H-NMR spectra on a Varian 300CXP apparatus (chemical shifts are expressed as δ values relative to Me_4Si as standard). Elemental analysis was done on a Carlo Erba Instrument CHNS Elemental Analyzer, Model 1106. Analytical HPLC was performed on a reversed-phase C_{18} Bondapack column, with a Beckman EM-1760 instrument. All reactions were monitored by thin-layer chromatography (TLC) using 0.25-mm precoated silica gel plates (E. Merck, Darmstadt, Germany). Compounds **6** were prepared by reaction of sodium N,N-dimethyl/diethyl-dithiocarbamates (from E. Merck) with arylsulfonyl halides (commercially available from Sigma-Aldrich, Fluka or E. Merck). Cysteine, glutathione, cystine, oxidized glutathione, colchicine and bovine brain tubulin were from Sigma-Aldrich (Milan, Italy). Solvents used were doubly distilled and kept on molecular sieves in order to maintain them in anhydrous conditions.

General Procedure for the Preparation of Derivatives 6

10 mMoles of sodium N,N-dialkyl-dithiocarbamates **7** or **8** dissolved in 50 mL of water were treated with the stoichiometric amount of arylsulfonyl halide dissolved in 25–50 mL of acetone. The reaction mixture was magnetically stirred at room temperature for 4–15 h (TLC control) untill all the sulfonyl halide was converted, the solvent (acetone) was evaporated *in vacuo*, and the precipitated arylsulfonyl-N,N-dialkyl-dithiocarbamates filtered and recrystallized from acetone. Yields were almost quantitative in all cases. All compounds were fully characterized by means of IR, ^{1}H-NMR, ^{13}C-NMR spectral and elemental analysis data, which confirmed the proposed structures. Data for several representative compounds are shown below.

4-Iodophenylsulfonyl-N,N-dimethyldithiocarbamate, **6e** As tan crystals, m.p 160–2 °C (dec.). IR (KBr), cm^{-1}: 1150 (SO_2^{sym}), 1312 (thioamide III), 1377 (SO_2^{as}), 1540 (thioamide II), 1744 (thioamide I). ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.43 (s, 6H, NMe_2), δ_A 7.09, δ_B 7.78 (AA'BB'system, 4H, $J_{AB} = 8.1$ Hz,ArH); ^{13}C-NMR(DMSO-d_6),δ, ppm: 34.3 (s, Me_2N), 129.1, 132.8, 135.6, 142.8, 175.5 (C=S). Found: C, 27.85; H, 2.37; N, 3.47; S, 25.12. $C_9H_{10}INO_2S_3$ requires: C, 27.90; H, 2.60; N, 3.62; S, 24.84%.

4-Carboxyphenylsulfonyl-N,N-dimethyldithiocarbamate, **6j** As tan crystals, m.p 136–8 °C (dec.). IR (KBr), cm^{-1}: 1155 (SO_2^{sym}), 1316 (thioamide III), 1374 (SO_2^{as}), 1540 (thioamide II), 1740 (thioamide I). ^{1}H-NMR (DMSO-d_6), δ, ppm: 3.38 (s, 6H, NMe_2), δ_A 7.15, δ_B 7.73 (AA'BB'system, 4H, $J_{AB} = 7.9$ Hz, ArH), 10.23 (br s, 1H, COOH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 34.9 (s, Me_2N), 129.3, 132.7, 135.9, 142.8, 176.0 (C=S), 182.5 (COOH). Found: C, 39.21; H, 3.54; N, 4.37; S, 31.64. $C_{10}H_{11}NO_4S_3$ requires: C, 39.33; H, 3.63; N, 4.59; S, 31.50%.

4-Fluorophenylsulfonyl-N,N-diethyldithiocarbamate, **6l** As tan crystals, m.p 177–80 °C (dec.). IR (KBr), cm^{-1}: 1148 (SO_2^{sym}), 1315 (thioamide III), 1353 (SO_2^{as}), 1546 (thioamide II), 1742 (thioamide I). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.72 (t, 6H, $^3J_{HH} = 6.4$ Hz, 2Me from NEt_2), 3.85 (m, 4H, 2 CH_2 of Et_2N), δ_A 7.19, δ_B 7.74 (AA'BB'system, 4H, $J_{AB} = 8.0$ Hz, ArH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 25.6 (s, CH_3 of Et_2N), 43.5 (s, CH_2 of Et_2N), 130.3, 132.6, 134.8, 145.9, 175.6 (C=S). Found: C,

42.67; H, 4.25; N, 4.49; S, 31.45. $C_{11}H_{14}FNO_2S_3$ requires: C, 42.98; H, 4.59; N, 4.56; S, 31.29%.

4-Methylphenylsulfonyl-N,N-diethyldithiocarbamate **6p** As white crystals, m.p 154–6 °C (dec.). IR (KBr), cm^{-1}: 1159 (SO_2^{sym}), 1315 (thioamide III), 1365 (SO_2^{as}), 1541 (thioamide II), 1746 (thioamide I). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.50 (s, 3H. Me of tosyl), 2.74 (t, 6H, $^3J_{HH} = 6.4$ Hz, 2Me from NEt_2), 3.83 (m, 4H, $2CH_2$ of Et_2N), δ_A 7.11, δ_B 7.81 (AA'BB'system, 4H, $J_{AB} = 8.0$ Hz, ArH); ^{13}C-NMR (DMSO-d_6), δ, ppm: 23.5 (s, CH_3 of tosyl), 25.5 (s, CH_3 of Et_2N), 43.2 (s, CH_2 of Et_2N), 130.2, 133.8, 138.1, 145.4, 175.0 (C=S). Found: C, 47.55; H, 5.78; N, 4.26; S, 32.03. $C_{12}H_{17}NO_2S_3$ requires: C, 47.50; H, 5.65; N, 4.59; S, 31.70%.

Reaction of arylsulfonyl-N,N-dialkyl-dithiocarbamates with Thiol Reagents

5 mL of a 0.1 mM solution of arylsulfonyl-N, N-dialkyl-dithiocarbamate in 10 mM phosphate buffer (pH 7.4) with 10% DMSO (v/v) and 5 mL of 0.1 mM solution of cysteine (Cys) or glutathione (Glt) in the same buffer (without DMSO) was incubated at 37 °C for 24 h. By means of HPLC, the amount of transformed Cys/Glt was followed (C_{18} reversed-phase μ-Bondapack (2.5 × 150 mm) column; 90% phosphate buffer/10% acetonitrile, 3 mL/min). Authentic cysteine and glutathione were used as HPLC standards (retention times R_T of 5.45 min for Cys and 7.80 min for Glt under the conditions of the experiment). The same experiments were also performed with cystine (Cys-Cys, R_T of 6.83 min) and oxidized glutathione (R_T of 8.63 min) instead of the two thiol reagents mentioned above, when no chemical modifications could be seen. Blank experiments were also performed by incubating the arylsulfonyl-N, N-dialkyl-dithiocarbamate solution (as above) with phosphate buffer without the thiol reagent/disulphides mentioned above. In these cases too, no reaction could be seen, and the starting arylsulfonyl-N,N-dialkyl-dithiocarbamate was isolated unchanged after the incubation, proving that these compounds are stable enough in solution (at least for 24 h). DMSO was present in the arylsulfonyl-N,N-dialkyl-dithiocarbamate solution due to the poor solubility of these compounds in water/buffer.

Inhibition of Tumor Cell Growth

Stock solutions of arylsulfonyl-N,N-dialkyl-dithiocarbamate (1 mM) were prepared in DMSO, and dilutions up to 10 nM were done 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)[16]:

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

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

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 h exposure of cells to the test compounds; Mean OD_{ctrl} = the average optical density measurements of SRB-derived color after 48 h with no exposure of cells to the test compounds.

GI_{50} represents the molarity of compound 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%.[16] The standard suforhodamine B (SRB) protein assay was used to estimate cell viability or growth.[16]

In vitro Tubulin Polymerization Assay

The *in vitro* tubulin polymerization reaction was assayed as described in references.[9a,15,17] Ice-cold bovine brain tubulin solution (400 μg protein 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 **6**, or colchicine, or **2a** X = F; Y = OMe). The mixture was transferred to a quartz cuvette equilibrated at 37 °C. Changes in the optical density (OD) at 340 nm were registered with a Perkin Elmer spectrophotometer every 30s for 10 min, maintaining the temperature at 37 °C. Colchicine used as standard inhibitor was from Sigma Chemical Co. (Milan, Italy), whereas compound **2a** also used as standard was synthesized from pentafluorophenylsulfonyl chloride and *p*-anisidine, as described in references.[5–7] The turbidity of the test solutions with or without tubulin polymerization inhibitors were plotted on a linear scale *versus* time, for the 10 min periods during which these reactions were followed.

RESULTS AND DISCUSSION

Reaction of arylsulfonyl halides with sodium N,N-dialkylditiocarbamates (in acetone-water) afforded a series of arylsulfonyl-N,N-dialkyl-dithiocarbamates **6a–t** (Table I). All these compounds were characterized by spectral and elemental analysis data that confirmed their structure.

The reaction of the new derivatives **6** with cysteine (Cys) and glutathione (Glt) was investigated, by means of HPLC, by incubating equimolar amounts of **6** and Cys/Glt in phosphate buffer at 37 °C for 24 h, in order to identify compounds that should readily react with the Cys residues of β tubulin. The amount of labeled Cys/Glt is shown in Table I. It can be seen that the N,N-diethyl-derivatives were more reactive than the corresponding N,N-dimethyl-substituted compounds. In the sub-series of arylsulfonyl-N,N-dimethyl/diethyl-dithiocarbamates the weakest reactivity was observed for the unsubstituted phenyl derivatives **6a** and **6k**, respectively, whereas substitution with methyl, fluorine or chlorine atoms in the 4-position of the aromatic ring dramatically increased the reactivity. For the diverse halogeno-substituted compounds, reactivity was best with the fluoro- and chloro-substituted compounds, but tended to decrease for the bromo- and iodo-substituted ones. The same effect has been observed for the 4-methoxy- or 2,4,6-trimethyl-substituted compounds as compared to the 4-methylphenyl-substituted derivatives **6f** and **6p**, respectively. 4-Carboxyphenyl-substituted derivatives were also more reactive than the isomeric 3-carboxy-

TABLE I Arylsulfonyl-N,N-dialkyl-dit hiocarbamates **6a–t**, and their reactivity against thiol reagents (cysteine, Cys, and Glutathione, Glt). $RSO_2SC(=S)NMe_2$ (**6a–j**), $RSO_2SC(=S)NEt_2$ (**6k–t**)

Compound 6	R	Synthetic method	%SH modification*	
			Cys	Glt
a	Ph	A	10	3
b	*p*-F-C_6H_4–	A	62	54
c	*p*-Cl-C_6H_4–	A	64	60
d	*p*-Br-C_6H_4–	A	55	49
e	*p*-I-C_6H_4–	A	44	28
f	*p*-Me-C_6H_4–	A	61	60
g	*p*-MeO-C_6H_4–	A	34	21
h	2,4,6-$Me_3C_6H_2$–	A	18	16
i	*m*-HOOC-C_6H_4–	A	47	43
j	*p*-HOOC-C_6H_4–	A	59	61
k	Ph	B	17	10
l	*p*-F-C_6H_4–	B	79	66
m	*p*-Cl-C_6H_4–	B	81	76
n	*p*-Br-C_6H_4–	B	60	52
o	*p*-I-C_6H_4–	B	49	37
p	*p*-Me-C_6H_4–	B	85	74
q	*p*-MeO-C_6H_4–	B	44	36
r	2,4,6-$Me_3C_6H_2$–	B	20	20
s	*m*-HOOC-C_6H_4–	B	48	45
t	*p*-HOOC-C_6H_4–	B	60	57

A – Sodium N,N-dimethyldithiocarbamate (**7**) + RSO_2Cl; B – Sodium N,N-diethyldithiocarbamate (**8**) + RSO_2Cl.
*After 24 h incubation in 1:1 molar ratio of the reagents, in phosphate buffer (see Material and Methods).

phenyl-substituted derivatives. All these compounds generally reacted more rapidly with cysteine than with glutathione.

Several of the most reactive arylsulfonyl-N, N-dialkyl-dithiocarbamates **6** (such as **6j**, **6l**, **6m**, **6p**) against the thiol reagents mentioned above were then chosen to be tested for their tumor growth inhibitory properties. The obtained data are shown in Table II. The tumor cell growth inhibition measurements were done by the NIH National Cancer Institute, Bethesda, MD, USA, on our behalf. The following cancer cell types were included in these assays: leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer and prostate and breast cancer.

The following should be noted regarding the tumor cell growth inhibition data with the test compounds **6**: (i) different cancer cell lines of the same tumor type, possessed a very variable response to inhibition of growth in the presence of the new derivatives. For example, the SR leukemia cells were very susceptible to inhibition by **6m** or **6p** (GI_{50} of 20–70 nM), whereas other leukemia cell lines (such as HL-60, K-562, MOLT-4) showed the same level of inhibition only at concentrations between 20–33 μM of inhibitor. The same situation was seen in the case of diverse non-small cell lung cancer cell lines, with **6l, 6m** or **6p** acting as very potent inhibitors (GI_{50} in the range of 10–50 nM) against the NCI-H522 line whereas the related HOP-62 line showed the same level of inhibition at concentrations as high as 22–40 μM. Other cell lines of this tumor, such as A549/ATCC, had an intermediate behavior between the two extremes reported above ($GI_{50} = 0.4$ μM, with compound **6m**); (ii) all the investigated cancer lines were generally inhibited by one of the compounds tested, but some types of tumors such as the leukemia or non-small cell lung ones were generally more susceptible to inhibition, whereas others, such as the colon, renal CNS, melanoma, ovarian, 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 **6** with GI_{50} values in the nanomolar range. These included the SR leukemia with **6l, 6m** and **6p**, CCRF-CEM leukemia with **6p** and NCI-H522 non-small cell lung cancer with all the three derivatives mentioned above (Table II). The largest majority of susceptible tumors were generally inhibited at micromolar concentrations of the test compounds with GI_{50} values in the range of 4–60 μM; (iv) important differences of activity between the investigated compounds **6** were detected, with the 4-carboxy-phenyl-substituted derivative **6j** showing poor antitumor activity, whereas the 4-halogeno- or 4-methylphenyl-substituted compounds possessed broad tumor inhibitory properties against many of the investigated tumor cell lines; (v) the inhibition of growth of tumors 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-dialkyldithiocarbamate.

The mechanism of tumor growth inhibition with these arylsulfonyl-N,N-dialkyl-dithiocarbamates is not known at present but a hypothesis may be made in this regard. Taking into account the fact that we showed that some of these derivatives may modify thiol reagents such as cysteine and glutathione (but not cystine or oxidized glutathione, see Experimental) we hypothesize that their cellular target is tubulin. This hypothesis has been verified by performing tubulin polymerization experiments in the presence of well known inhibitors such as colchicine,[9,17] or the recently described pentafluorophenylsulfonamide **2a** (X = F; Y = OMe)[5–7] as well as some of the new compounds of type **6** described in the present paper (Figure 1).

It may be seen that both colchicine (at a concentration of 3 μM – curve 3) as well as the strong tubulin polymerization sulfonamide inhibitor **2a**[6–9a] (at the same concentration – curve 5) strongly inhibited microtubule formation (in curve 1, the turbidity was measured with no drug added). The same behavior as for colchicine or **2a** has

TABLE II *In vitro* tumor growth inhibition data for some of the new compounds **6** synthesized in the present work

Tumor	cell line	GI_{50} (μM)*			
		6j	**6l**	**6m**	**6p**
Leukemia	HL-60 (TB)	>100	–**	–	33
	MOLT-4	>100	26	18	20
	K-562	>100	23	14	28
	SR	>100	0.3	0.07	0.02
	CCRF-CEM	82	–	–	<0.01
	RPMI-8226	–	17	15	21
Non-small	NCI-H23	>100	76	38	42
cell lung	HOP-62	>100	40	22	23
cancer	HOP-92	>100	31	17	19
	NCI-H460	>100	43	29	27
	NCI-H522	4	0.05	0.03	<0.01
	A549/ATCC	–	–	0.4	>100
Colon	COLO-205	>100	>100	26	63
cancer	HCT-15	>100	66	31	36
	HT29	>100	22	17	13
	SW-620	>100	18	17	20
	HCT-116	>100	25	30	21
	KM12	>100	17	11	14
CNS	SF-268	>100	>100	53	55
cancer	SF-295	>100	75	38	52
	SF-539	–	23	21	17
	SNB-19	>100	>100	38	61
	U251	>100	18	15	23
Melanoma	LOX IMVI	>100	19	15	17
	M14	>100	29	17	14
	MALME-3M	–	20	11	46
	UACC-257	>100	28	24	20
	SK-MEL-28	>100	14	18	18
	SK-MEL-2	>100	56	66	50
	UACC-62	–	20	20	16
Ovarian	OVCAR-5	>100	>100	44	>100
cancer	OVCAR-4	>100	>100	45	43
	OVCAR-3	>100	>100	31	39
	OVCAR-8	>100	32	17	>100
Renal	768–0	>100	73	23	20
cancer	ACHN	>100	43	33	34
	CAKI-1	>100	41	19	17
	RXF 393	>100	18	17	16
	UO-31	>100	27	17	20
	TK-10	–	>100	68	>100
	SN12C	–	34	20	17
Prostate	PC-3	>100	–	–	–
cancer	DU-145	>100	38	27	42
Breast	MCF7	>100	54	21	39
cancer	MDA-MB-231/ATCC	>100	32	35	35
	NCI/ADR-RES	>100	50	35	36
	MDA-N	>100	63	36	28
	HS 578T	>100	63	43	75
	MDA-MB-435	>100	39	20	31
	BT-549	>100	46	34	28
	T-47D	–	85	46	60

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

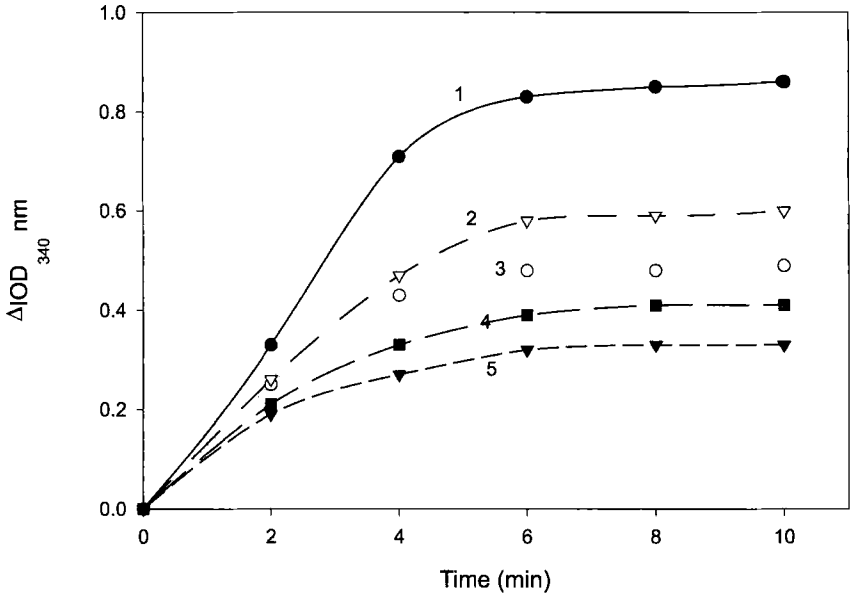
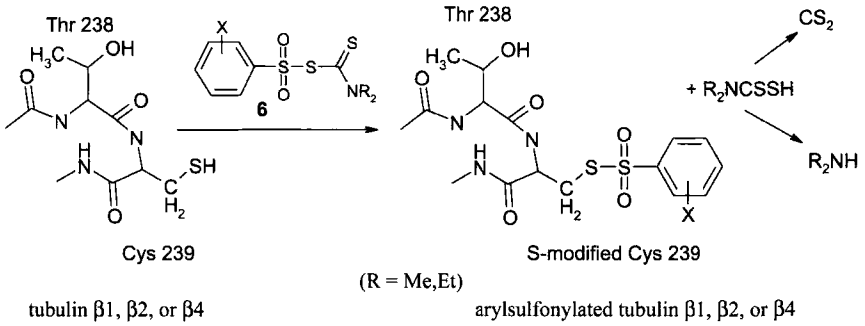


FIGURE 1 *In vitro* tubulin polymerization turbidimetric assay. Changes of OD at 340 nm with time: curve 1 – with no drug added; curve 2–3 μM compound **6l**; curve 3–3 μM colchicine; curve 4–3 μM compound **6p**; curve 5–3 μM compound **2a**.

been observed with the new derivatives **6l** and **6p** reported in the present paper. At a concentration of 3 μM (curve 2), compound **6l** was less inhibitory than colchicine, whereas the structurally related derivative **6p** (curve 4) was more inhibitory than colchicine but slightly less inhibitory than the pentafluorophenylsulfonamide **2a** which is a very potent microtubule formation inhibitor.[6–9a]

Thus, in a similar manner to sulfonamides **2** investigated in detail by Medina's group,[6–9a] the compounds **6** reported here probably modify the SH moiety of Cys 239 of tubulin β1, 2 or 4 isotypes, leading thus to disruption of cellular microtubules. A possible labeling of tubulin by one of the arylsulfonyl-N,N-dialkyl-dithiocarbamates reported here is shown schematically in Scheme 1.

It should also be noted that in a very recent patent,[18] some compounds structurally related to those described here (of the general formula $[R_2N\text{-}CSS)]_2$; R = alkyl; cycloalkyl) were shown to be effective in the treatment or prophylaxis of viral diseases provoked (or exacerbated) by mammalian papilloma viruses (PVs). Human PVs on the other hand are known to be asso-



SCHEME 1

ciated with malignant neoplasias among which is cervical cancer.[18] The mechanism by which these compounds ($[R_2N\text{-}CSS)]_2$) inhibit viral/tumor replication is not known at present, but it was suggested that it may involve their reaction with some cysteine residues of a viral zinc finger protein.[18] Thus, it is possible that compounds such as those described here, containing moieties able to react with critically important cysteine residues of different target proteins, could lead to new types of therapeutcally useful compounds for the treatment or prevention of viral/cancerous diseases.

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