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Bioorganic & Medicinal Chemistry
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Bioorganic & Medicinal Chemistry 12 (2004) 963–968

Synthesis and cytotoxic activity of lipophilic sulphonamide derivatives of the benzo[*b*]thiophene 1,1-dioxide

R. Villar,[a] I. Encio,[b,*] M. Migliaccio,[b] M. J. Gil[a] and V. Martinez-Merino[a,*]

[a]*Dpto. de Química, Universidad Pública de Navarra, Campus Arrosadía, 31006 Pamplona, Spain*
[b]*Dpto. de Ciencias de la Salud, Universidad Pública de Navarra, Avda. Barañain, 31008 Pamplona, Spain*

Received 22 October 2003; accepted 12 December 2003

Abstract—In the search of new compounds with antineoplastic activity, we have analysed the effect of several structural modifications on the nucleus 6-benzo[*b*]thiophenesulphonamide 1,1-dioxide on its cytotoxic activity on tumour cells. Lipophilic substituents on the sulphonamide group significantly increased the cytoxic activity measured using a panel of human tumour cell lines. Only slight variations on cytotoxicity were obtained when the sulphonamide group occupied the position 5 of the system. The most active compound was the *N*-4-methoxyphenyl derivative **15**, which showed GI_{50} values of 1–9 nM against HT-29, CCRF-CEM, K-562 and MEL-AC cells and of 200 nM against HTB-54 cells. Free access to the 3-position of the heterocyclic system seems to be required to obtain cytotoxic derivatives. Derivative **15** was also active at the same level of commercial Doxorubicine against cultured normal human lung fibroblasts.

1. Introduction

A series of benzo[*b*]thiophenesulphonamide 1,1-dioxide derivatives (BTS) were recently reported as a new class of potential antineoplastic agents.[1] These compounds showed a clear correlation between their cytotoxic activities and their ability to inhibit an specific NADH oxidase located at the membrane of leukaemia CCRF-CEM cells.[2] Moreover, unsubstituted BTS and their corresponding sulphonylurea derivatives induced an apoptotic process in CCRF-CEM cells that is mediated by the generation of reactive oxygen species (ROS), and includes typical apoptotic features such as cell shrinkage, PS translocation to the cell surface, mitochondrial dysfunction, caspase activation, chromatin condensation and internucleosomal DNA degradation.[3] Thus, these compounds may exert its effect by affecting the activity of enzymes related with the control of cellular ROS levels. Up to now, unsubstituted BTS showed better properties as antitumour agents than the corresponding benzo[*b*]thiophenesulphonylureas, which were developed from antineoplastic diarylsulphonylureas[4] by a 3D-QSAR model.[5,6] Despite of the interesting characteristics described above, cytotoxicity of the lead compound **1** (Fig. 1) against tumour cells was observed at doses moderately high (GI50 > 2 μM). Therefore, we decided to look for new derivatives with stronger antineoplastic activity.

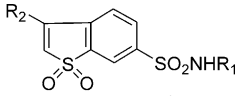

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Figure 1.

Some enzymes related with ROS control such as the glutathione reductase and the glutathione *S*-transferase, present a hydrophobic pocket near their active site.[7,8] Thus, new benzothiophene 1,1-dioxide derivatives **6–20** were designed carrying hydrophobic substituents of different length and grade of flexibility on the sulphonamide group. Moreover, some of them possess groups with capacity to form hydrogen bonds. Additionally, to analyse different distances between polar groups, the sulphonamide group was inserted at the positions 5 and 6 of the benzo[*b*]thiophene nucleus (Scheme 1). Since the reaction of the thiol groups at the position 3 of the system was previously pointed out[2] as a determinant

Keywords: Benzo[*b*]thiophenesulphonamide 1,1-dioxide derivatives; Cytotoxic activity; Synthesis; Structure–activity relationships; Lipophilicity.
* Corresponding author. Tel.: +34-948-169590; fax: +34-948-169606; e-mail: merino@unavarra.es

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doi:10.1016/j.bmc.2003.12.012

factor in the cytotoxic activity of this kind of compounds,[2] the series also explores substituents that would hinder such reaction. Our results demonstrate that some of the synthesised derivatives of the benzo[*b*]thiophene 1,1-dioxide presented a strong, highly increased cytotoxic activity on human colon carcinoma (HT-29), lung carcinoma (HTB-54), lymphocytic leukaemia (CCRF-CEM), myelocytic leukaemia (K-562) and melanoma (MEL-AC) cell lines.

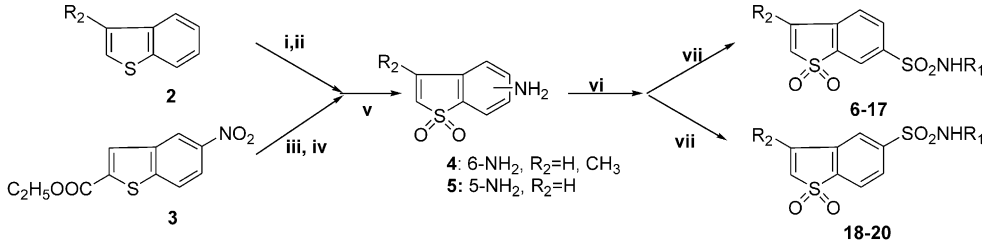
2. Chemistry

BTS derivatives **6–20** were prepared as shown in Scheme 1. The oxidation of benzo[*b*]thiophene **2** ($R_2 = H$, CH_3) with 30% hydrogen peroxide, and the subsequent nitration of the 1,1-dioxide intermediate by the Challenger's method[9] using nitric acid (d = 1.52 g/mL) gave the corresponding 6-nitrobenzo[*b*]thiophene 1,1-dioxides (60% yield). The latest compounds were reduced to 6-aminobenzo[*b*]thiophene 1,1-dioxide **4** ($R_2 = H$, CH_3) with iron-ammonium chloride (87% yield). The 5-amino isomer **5** was prepared from the ethyl 5-nitro-2-benzo[*b*]thiophenecarboxylate **3**, which was obtained following the method described by Rossi and Trave.[10] Compound **3** was hydrolysed and then decarboxylated by heating in cooper/quinoline under nitrogen atmosphere to give the 5-nitrobenzo[*b*]thiophene (90% yield), which was then oxidised to its 1,1-dioxide by using 3-chloroperoxybenzoic acid, and the nitro group selectively reduced with iron-ammonium chloride to give the 5-aminobenzo[*b*]thiophene 1,1-dioxide **5** (52% yield). The amino derivatives **4** and **5** were transformed into the corresponding chlorosulphonyl derivatives using the Meerwein's method[11] (treatment of diazonium salts with sulphonyl chloride in the presence of cuprous chloride), and then treated with either ammonia or amines to give the 6-benzo[*b*]thiophenesulphonamide 1,1-dioxide derivatives **6–17** (12-95% yield) or the 5-benzo[*b*]thiophenesulphonamide 1,1-dioxide derivatives **18–20** (30–72% yield) respectively. Spectroscopic properties of all compounds were in agreement with their structures.

3. Results and discussion

Cytotoxic activity of the lead compound **1** and sulphonamides **6–20** against five human tumour cell lines are displayed in Table 1. All compounds were tested in the range of 0.01-100 μM, or lower when GI_{50} was inferior to 10 nM.[12] As shown in Table 1, though CCRF-CEM and MEL-AC cells were generally more sensitive and K-562 more resistant to the cytotoxic effect, growth of every cell line was clearly affected by all the tested compounds and it is worth noting that all compounds were more cytotoxic against each one of the tested cell lines than the lead compound **1**. Figure 2 shows exemplary curves with the original data from which the GI_{50} values for compounds **7**, **10**, **13** and **15** in K-562 and HT-29 cells were calculated.

The influence of the sulphonamide position on cytotoxicity seems to be weak. When 5 and 6 BTS's were



Scheme 1. (i) Acetic acid, H_2O_2 30% (v/v), reflux, 30 min; (ii) nitric acid 100%; (iii) NaOH, Ethyl alcohol/water 50%; Cu/Quinolin 200 °C; (iv) MCPBA; (v) Fe/NH_4Cl, ethanol/H_2O 50% (v/v); (vi) $NaNO_2$, HCl (ac); SO_2/CuCl, Acetic acid; (vii) NH_2R, dioxane.

Table 1. Cytotoxic activities (GI_{50}, μM inhibition of cell growth) against tumoural cell lines and lipophilicity (*logP*) of BTS

Compd	R_1	R_2	HT-29	HTB-54	CCRF-CEM	K-562	MEL-AC	*LogP*
1	H	H	8.55	5.89	2.86	29.41	7.14	*−0.03*
6	C_2H_5	H	2.80	0.06	2.11	6.57	3.00	*1.25*
7	C_4H_9	H	0.60	0.79	0.08	0.81	0.73	*2.31*
8	$CH_2C_3H_5$	H	0.51	N.D.[a]	0.21	4.16	2.00	*1.70*
9	C_6H_{13}	H	1.46	N.D.[a]	3.3	2.48	0.06	*3.37*
10	$CH_2C_6H_5$	H	0.80	0.72	0.28	0.71	0.39	*2.49*
11	$C_2H_4OCH_3$	H	5.87	2.50	3.30	4.86	5.49	*0.77*
12	C_4H_8OH	H	0.47	1.38	3.13	20.68	4.46	*0.33*
13	$C_3H_6COOC_2H_5$	H	1.51	0.90	2.70	2.39	2.89	*1.91*
14	$C_4H_8COOC_2H_5$	H	2.79	1.92	0.72	2.09	1.12	*1.80*
15	*p*-$C_6H_4OCH_3$	H	0.007	0.20	0.005	0.001	0.009	*2.34*
16	*m*-C_6H_4Cl	H	0.29	N.D.[a]	0.21	0.46	0.18	*3.16*
17	H	CH_3	> 100	> 100	> 100	> 100	> 100	*0.49*
18	C_4H_9	H	2.84	0.49	2.78	2.67	0.78	*2.31*
19	$CH_2C_6H_5$	H	2.38	0.14	1.58	1.26	0.27	*2.49*
20	*m*-$C_6H_4OCH_3$	H	4.06	0.57	0.23	1.41	0.44	*2.34*

[a] Not determined.

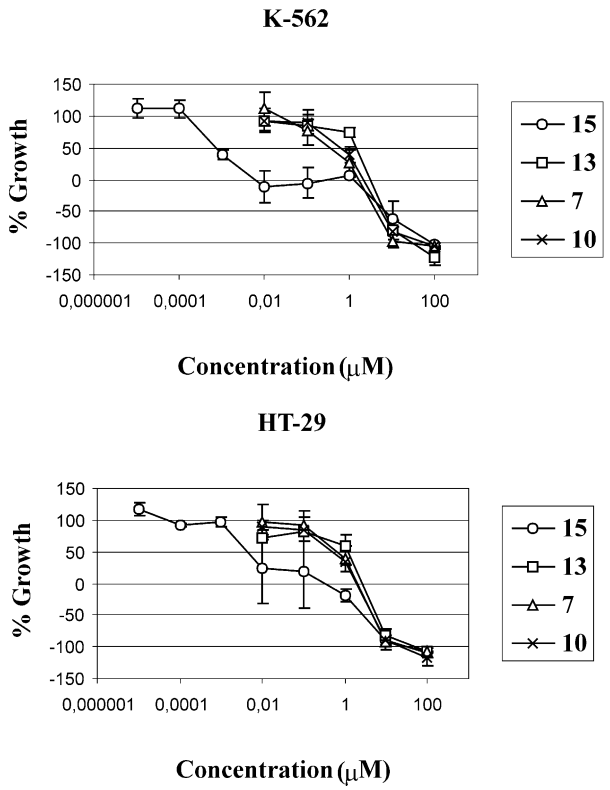


Figure 2. Determination of the cytotoxic effect of BTS **7**, **10**, **13** and **15** on K-562 and HT-29 cells. Cells were incubated in the presence of every compound at the indicated concentration for 72 h. Cytotoxicity was then determined by a colorimetric microassay based on the use of MTT[12]. Values represent means ± SD derived from 3 independent experiments each performed in quadruplicate.

compared (**7** vs **18**, **10** vs **19** and, in less degree **16** vs **20**), their cytotoxic activities were within the same order of magnitude, except for the butyl derivative against CCRF-CEM cells (Table 1).

On the other hand, the only presence of a methyl group at position 3 of the heterocyclic system, as in compound **17**, abolished the cytotoxic activity against all tested cell lines. This fact could be related with a steric hinder on 3-position of BTS towards the thiol reactivity at the active site of a protein involved in the ROS response. The lack of cytotoxicity of the 2,3-dihydro-6-benzo[*b*]-tiophenesulphonamide 1,1-dioxide,[2] a compound with low reactivity at position 3 with thiol groups, further supports this idea. To confirm this hypothesis, additional studies about the mode of action of BTS's are under progress.

In some cases, a clear correlation could be observed between the lipophilicity (logP)[13] and the cytotoxic activity of the active compounds (pGI_{50} in Fig. 3). The observed correlation most probably reflects the interaction with a target molecule rather than a transport phenomenon. However, since the 4-methoxyphenyl derivative **15** was strongly active, other properties than lipophilicity should be involved in the cytotoxicity of these compounds, as it has been pointed above. When the compound **15** was excluded from the statistical analysis, leave-one-out square correlation coefficient

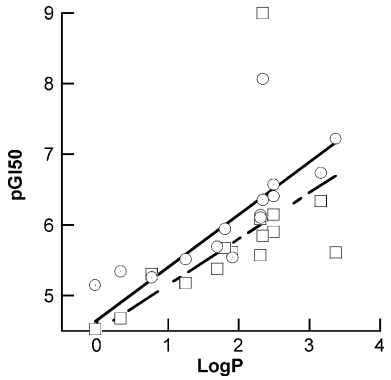


Figure 3. BTS's Cytotoxicities ($pGI_{50} = -\log GI_{50}$) against MEL-AC (○) and K-562 (□) cells vs logP.

(q^2) increased from 0.11 to 0.62 for the linear relation of K562 pGI_{50} vs logP, and from 0.51 to 0.81 for the linear relation of MEL-AC pGI_{50} vs logP.

BTS **15** was as active as commercial Doxorubicine both against CCRF-CEM and MEL-AC tumour cells ($GI_{50} = 33$ nM and 6 nM respectively), and normal human lung fibroblasts growing in culture ($GI_{50} = 8$ nM for **15** and 6 nM for Doxorubicine).

In conclusion, BTS are a novel class of potential antitumour agents that includes compounds as cytotoxic as some commercial drugs. BTS **15** could be a new lead compound where it should be possible to introduce structural modifications to gain specificity towards tumour cells.

4. Experimental

4.1. General

Melting points were determined in a Mettler FP82HT + FP80 apparatus and are uncorrected. Routine monitoring of reactions was performed using Alugram SilG/UV_{254} (0.20 mm). All chromatographic separations were performed using silica gel (Merck 60 230–400 mesh). ^{1}H NMR spectra were recorded on a Varian 200 MHz spectrometer with TMS as internal standard. Chemical shifts are reported in ppm and coupling constants in Hz. IR spectra of precursors were recorded on an Avatar 360 FT-IR spectrophotometer. Elemental analyses were carried out on a Carlo Erba EA1108 elemental analyser from vacuum-dried samples (over phosphorus pentoxide at 3–4 mmHg, 6–12 h at about 30–70 °C).

4.1.1. General procedure for the synthesis of the benzo[*b*]thiophenesulphonamide 1,1-dioxide derivatives (6–20). To a stirred solution of the corresponding aminobenzo[*b*]thiophene 1,1-dioxide **4**, **5** (1.84 mmol) in hydrochloric acid (37% v/v, 6 mL) and glacial acetic acid (3 mL) at −10 °C, a solution of 0.13 g (1.9 mmol) of $NaNO_2$ in 1 mL of water was drop wise added, and the stirring was continued at room temperature for 1 h. That mixture was cooled at 0 °C and then drop wise

added to a stirred mixture of 0.043 g (0.43 mmol) of CuCl in glacial acetic acid (3 mL) at 0 °C previously saturated with SO_2. The resulting mixture was furthermore stirred at room temperature for 1 h. Cold water and ice (250 g) were added and the obtained solid was collected and dried to give the corresponding chlorosulphonyl derivative which was used without further purification. To a solution of the above chlorosulphonyl derivative (1.84 mmol) in dioxane (5 mL), a solution of the appropriate amine (1.84 mmol) and triethylamine (1.84 mmol) in dioxane (5 mL) was added with stirring. (Compound **17** was prepared by passing ammonia gas through a stirred solution of the 6-chlorosulphonyl derivative). Stirring was continued for 30–60 min at room temperature. Solvents were removed in vacuum. The residual material was dissolved in dichloromethane (100 mL), washed successively with 5% HCl (2×50 mL) and saturated aqueous $NaHCO_3$ (2×50 mL), dried over $MgSO_4$ and evaporated. The solid was purified by crystallization or by chromatography.

In this manner, the following products were obtained.

4.1.2. *N*-ethyl-6-benzo[*b*]thiophenesulphonamide 1,1-dioxide (6). Starting from 6-chlorosulfonylbenzo[*b*]thiophenesulphonamide 1,1-dioxide and ethylamine hydrochloride, compound **6** was obtained and recrystallised from ethanol (36%): Mp 162.5–163.5 °C; IR (HATR cm^{-1}) 1149, 1300 (SO_2), 3281 (NH). ^1H NMR ($CDCl_3$ δ): 8.14 (s, 1H, H-7), 8.05 (d, $J_{54}=7.69$ Hz, 1H, H-5), 7.51 (d, 1H, H-4), 7.27 (d, $J_{32}=6.96$ Hz, 1H, H-3), 6.89 (d, 1H, H-2), 4.55 (t, 1H, NH), 3.05 (m, 2H, NH–$\underline{CH_2}$–); 1.14 (t, 3H, CH_3). Anal. calcd ($C_{10}H_{11}NO_4S_2$): C, 43.94; H, 4.06; N, 5.12; S, 23.46. Found: C, 44.01; H, 4.27; N, 5.04; S, 24.11.

4.1.3. *N*-butyl-6-benzo[*b*]thiophenesulphonamide 1,1-dioxide (7). Starting from 6-chlorosulfonylbenzo[*b*]thiophenesulphonamide 1,1-dioxide and butylamine, compound **7** was obtained and recrystallised from isopropanol (71%): Mp 117 °C; IR (HATR cm^{-1}): 1154, 1316 (SO_2), 3306 (NH); ^1H NMR ($CDCl_3$ δ): 8.19 (s, 1H, H-7), 8.09 (d, $J_{54}=7.69$ Hz, 1H, H-5), 7.55 (d, 1H, H-4) 7.32 (d, $J_{32}=6.96$ Hz, 1H, H-3), 6.93 (d, 1H, H-2), 4.52 (t, $J=5.86$ Hz, 1H, NH), 3.03 (m, 2H, NH–$\underline{CH_2}$–), 1.59–1.22 (m, 4H, –CH_2–CH_2–CH_3), 0.91 (t, $J=7.33$ Hz, 3H, –CH_3). Anal. calcd ($C_{12}H_{15}NO_4S_2$): C, 47.82; H, 5.02; N, 4.65; S, 21.28. Found: C, 48.29; H, 5.34; N, 4.63; S, 21.29; MS: 301, 258, 229, 165.

4.1.4. N-cyclopropylmethyl-6-benzo[*b*]thiophenesulphonamide 1,1-dioxide (8). Starting from 6-chlorosulfonylbenzo[*b*]thiophenesulphonamide 1,1-dioxide and aminomethylciclopropane hydrochloride, compound **8** was obtained and recrystallised from isopropanol (31%): Mp 161–162 °C; IR (KBr, cm^{-1}): 1146, 1311 (SO_2), 3287 (NH); ^1H NMR ($CDCl_3$ δ): 8.16 (s, 1H, H-7), 8.06 (d, $J_{54}=7.69$ Hz, 1H, H-5), 7.50 (d, 1H, H-4), 7.27 (d, $J_{32}=6.96$ Hz, 1H, H-3), 6.88 (d, 1H, H-2), 4.69 (t, $J=5.86$ Hz, 1H, –NH), 2.88 (m, 2H, –CH_2–), 0.93–0.82 (m, 1H), 0.54–0.45 (m, 2H), 0.16–0.89 (m, 2H). Anal. calcd ($C_{12}H_{13}NO_4S_2$): C, 48.14; H, 4.38; N, 4.68; S, 21.42. Found: C, 47.93; H, 4.14; N, 4.58; S, 20.18.

4.1.5. *N*-hexyl-6-benzo[*b*]thiophenesulphonamide 1,1-dioxide (9). Starting from 6-chlorosulfonylbenzo[*b*]thiophenesulphonamide 1,1-dioxide and hexylamine, compound **9** was obtained and recrystallised from isopropanol (30%): Mp 102–103 °C; IR (HATR, cm^{-1}): 1145, 1310 (SO_2), 3257 (NH); ^1H NMR ($CDCl_3$ δ): 8.15 (s, 1H, H-7), 8.05 (d, $J_{54}=8.06$ Hz, 1H, H-5), 7.50 (d, 1H, H-4), 7.27 (d, $J_{32}=6.96$ Hz, 1H, H-3), 6.88 (d, 1H, H-2), 4.49 (s, 1H, NH), 2.96 (m, 2H, NH–$\underline{CH_2}$–), 1.45 (m, 2H, NH–CH_2–$\underline{CH_2}$–), 1.22 (m, 6H, –$\underline{CH_2}$–CH_2–CH_2–CH_3), 0.84 (t, $J=6.22$ Hz, 3H, –$\underline{CH_3}$). Anal. calcd ($C_{14}H_{19}NO_4S_2$): C, 51.04; H, 5.81; N, 4.25; S, 19.47. Found: C, 51.20; H, 6.16; N, 4.26; S, 19.73.

4.1.6. *N*-benzyl-6-benzo[*b*]thiophenesulphonamide 1,1-dioxide (10). Starting from 6-chlorosulfonylbenzo[*b*]thiophenesulphonamide 1,1-dioxide and benzylamine, compound **10** was obtained and recrystallised from isopropanol (58,1%): Mp 120 °C; IR (KBr, cm^{-1}): 1322, 1151 (SO_2), 3275 (NH); ^1H NMR ($CDCl_3$ δ): 8.10 (s, 1H, H-7), 7.99 (d, $J_{54}=8.06$ Hz, 1H, H-5), 7.43 (d, 1H, H-4), 7.33–7.15 (m, 6H, H-3, C_6H_5), 6.87 (d, 1H, H-2), 5.01 (t, $J=5.86$ Hz, 1H, NH), 4.19 (d, 2H, –$\underline{CH_2}$–C_6H_5). Anal. calcd ($C_{15}H_{13}NO_4S_2$): C, 53.72; H, 3.91; N, 4.18; S, 19.12. Found: C, 53.22; H, 3.94; N, 4.17; S, 18.41.

4.1.7. *N*-(2-methoxy)ethyl-6-benzo[*b*]thiophenesulphonamide 1,1-dioxide (11). Starting from 6-chlorosulfonylbenzo[*b*]thiophenesulphonamide 1,1-dioxide and 2-methoxyethylamine, compound **11** was obtained and recrystallised from isopropanol (25,4%): Mp 126–128 °C; IR (KBr, cm^{-1}): 1151, 1301 (SO_2), 3299 (NH). ^1H NMR ($CDCl_3$ δ): 8.16 (s, 1H, H-7), 8.06 (d, $J_{54}=7.69$ Hz, 1H, H-5), 7.50 (d, 1H, H-4), 7.26 (d, $J_{32}=6.96$ Hz, 1H, H-3), 6.88 (d, 1H, H-2), 4.94 (t, $J=5.86$ Hz, 1H, NH), 3.42 (t, $J=5.13$, 2H, –CH_2O–), 3.21 (s, 3H, –CH_3), 3.17 (m, 2H, NH–$\underline{CH_2}$–CH_2). Anal. calcd ($C_{11}H_{13}NO_5S_2$): C, 43.55; H, 4.32; N, 4.62; S, 21.14. Found: C, 43.78; H, 4.39; N 4.59; S, 21.31.

4.1.8. *N*-(4-hydroxy)butyl-6-benzo[*b*]thiophenesulphonamide 1,1-dioxide (12). Starting from 6-chlorosulfonylbenzo[*b*]thiophenesulphonamide 1,1-dioxide and 4-amino-1-butanol, compound **12** was obtained and and purified by column chromatography (ethyl acetate) (44,6%): Mp 115.7–116.5 °C;. IR (KBr, cm^{-1}): 1146, 1310 (SO_2), 3200–3600 (bs, NH, OH); ^1H NMR ($CDCl_3$ δ): 8.12 (s, 1H, H-7), 8.01 (d, $J_{54}=8.06$ Hz, 1, H-5), 7.46 (d, 1H, H-4), 7.23 (d, $J_{32}=6.96$ Hz, 1H, H-3), 6.84 (d, 1H, H-2), 5.48 (t, $J=5.86$ Hz, 1H, NH), 3.58 (t, $J=5.86$, 2H, –$\underline{CH_2}$–OH), 2.97 (m, 2H, $NHCH_2$–), 1.90 (s, 1H, OH), 1.55 (m, 4H, –$\underline{CH_2CH_2}$–CH_2OH). Anal. calcd ($C_{12}H_{15}NO_5S_2$): C, 45.41; H, 4.76; N, 4.41; S, 20.21. Found: C, 45.33; H, 4.74; N 4.30; S, 21.31.

4.1.9. Ethyl 4-(6-benzo[*b*]thiophenesulphonamido 1,1-dioxide)butyrate (13). Starting from 6-chlorosulfonylbenzo[*b*]thiophenesulphonamide 1,1-dioxide and ethyl 4-aminobutyrate hydrochloride, compound **13** was obtained and and purified by column chromatography (hexane 2:3 ethyl acetate) (95%): Mp 61 °C; IR (KBr,

cm^{-1}): 1300, 1169 (SO_2), 1706 (COOEt), 3244 (NH); ^1H NMR ($CDCl_3$ δ): 8.18 (s, 1H, H-7), 8.08 (d, $J_{54}=7.69$ Hz, 1H, H-5), 7.54 (d, 1H, H-4), 7.31 (d, $J_{32}=6.96$ Hz, 1H, H-3), 6.93 (d, 1H, H-2), 5.28 (t, $J=5.86$ Hz, 1H, –NH–), 4.14 (q, $J=6.96$ Hz, 2H, OCH_2CH_3), 3.07 (m, 2H, –NH–CH_2–), 2.40 (t, $J=6.96$ Hz, 2H, –CH_2COO), 1.60 (m, 2H, –CH_2–CH_2–CO), 1.27 (t, 3H, –CH_3). Anal. calcd ($C_{14}H_{17}NO_6S_2$): C, 46.78; H, 4.77; N, 3.90; S, 17.84. Found: C, 46.87; H, 4.92; N, 3.88; S, 17.84.

4.1.10. Ethyl 5-(6-benzo[*b*]thiophenesulphonamido 1,1-dioxide)valerate (14). Starting from 6-chlorosulfonylbenzo[*b*]thiophenesulphonamide 1,1-dioxide and ethyl 5-aminovalerate hydrochloride, compound **14** was obtained and purified by column chromatography (hexane 4:7 ethyl acetate) (11,7%): Mp 77–79 °C; IR (KBr, cm^{-1}): 1147, 1308 (SO_2), 1721 (COOEt), 3293 (NH); ^1H NMR ($CDCl_3$ δ): 8.16 (s, 1H, H-7), 8.07 (d, $J_{54}=7.69$ Hz, 1H, H-5), 7.54 (d, 1H, H-4), 7.32 (d, $J_{32}=6.96$ Hz, 1H, H-3), 6.99 (d, 1H, H-2), 5.38 (t, $J=6.23$, 1H, NH), 4.11 (q, $J=6.96$ Hz, 2H, CH_2–CH_3), 2.98 (m, 2H, –NH–CH_2), 2.29 (t, $J=6.23$ Hz, 2H, –CH_2–COO–), 1.62 (m, 4H, –CH_2–CH_2), 1.26 (t, 3H, –CH_3). Anal. calcd ($C_{15}H_{19}NO_6S_2$): C, 48.24; H, 5.13, N, 3.75; S, 17.17. Found: C, 48.19; H, 5.59; N, 3.75; S, 17.09.

4.1.11. *N*-(4-methoxy)phenyl-6-benzo[*b*]thiophenesulphonamide 1,1-dioxide (15). Starting from 6-chlorosulfonylbenzo[*b*]thiophenesulphonamide 1,1-dioxide and *p*-anisidine, compound **15** was obtained and recrystallised from isopropanol (20%): Mp 160–161 °C; (KBr, cm^{-1}): 1146, 1308 (SO_2), 3258 (NH). ^1H NMR ($CDCl_3$ δ): 8.01 (s, 1H, H-7), 7.77 (d, $J_{54}=8.06$ Hz, 1H, H-5), 7.38 (d, 1H, H-4), 7.22 (d, $J_{32}=6.96$ Hz, 1H, H-3), 7.00–6.76 (m, 5H, H-2, C_6H_4), 6.35 (s, 1H, –NH–), 3.76 (s, 3H, O–CH_3). Anal. calcd ($C_{15}H_{13}NO_5S_2$): C, 51.27; H, 3.73; N, 3.99; S, 18.25. Found: C, 51.48; H, 3.97; N, 3.96; S, 18.61.

4.1.12. *N*-(3-chloro)phenyl-6-benzo[*b*]thiophenesulphonamide 1,1-dioxide (16). Starting from 6-chlorosulfonylbenzo[*b*]thiophenesulphonamide 1,1-dioxide and 3-chloroaniline, compound **16** was obtained and recrystallised from toluene (19%): Mp 152–153 °C; (KBr, cm^{-1}): 1143, 1297 (SO_2), 3256 (NH). ^1H NMR (DMSO-d_6 δ): 10.76 (s, 1H, NH), 8.11 (s, 1H, H-7), 8.04 (d, $J_{54}=7.69$ Hz, 1H, H-5), 7.76 (d, 1H, H-4), 7.71 (d, $J_{32}=6.96$ Hz, 1H, H-3), 7.48 (d, 1H, H-2), 7.40–7.00 (m, 4H, H-2′, H-4′, H-5′, H-6′). Anal. calcd ($C_{14}H_{10}NO_4S_2Cl$): C, 47.26; H,2.83, N, 3.94; S, 18.02. Found: C, 47.24; H, 2.76; N, 3.79; S, 18.66.

4.1.13. 3-methyl-6-benzo[*b*]thiophenesulphonamide 1,1-dioxide (17). Through a stirred solution of the 3-methyl-6-chlorosulfonylbenzo[*b*]thiophenesulphonamide 1,1-dioxide (1.84 mmol) in dioxane (15 mL) ammonia gas was passed for 10 min. After removal the solvent under vacuum, the solid was recrystallised from water to give 0.10 g of **17** (31%). Mp 212–213 °C; IR (KBr, cm^{-1}): 1168, 1280 (SO_2) 3275, 3369 (NH_2); ^1H NMR (DMSO-d_6 δ): 8.15 (s, 1H, H-7), 8.13 (d, 1H, H-5), 7.85 (d, 1H, H-4), 7.67 (s, 2H, –NH_2), 7.35 (s, 1H, H-2), 2.31 (s, 3H, –CH_3).

4.1.14. *N*-butyl-5-benzo[*b*]thiophenesulphonamide 1,1-dioxide (18). Starting from 5-chlorosulfonylbenzo[*b*]thiophenesulphonamide 1,1-dioxide and butylamine, compound **18** was obtained and recrystallised from isopropanol (65%): Mp 147 °C; (KBr, cm^{-1}): 1163, 1311 (SO_2), 3307 (NH). ^1H NMR ($CDCl_3$ δ): 8.06 (d, $J_{67}=8.06$, 1H, H-6), 7.89 (s, 1H, H-4), 7.88 (d, 1H, H-7), 7.31 (d, $J_{32}=6.96$, 1H, H-3), 6.49 (d, 1H, H-2), 4.59 (t, $J=5.86$, 1H, –NH), 3.02 (m, 2H, –NH–CH_2–), 1.58–1.21 (m, 4H, –CH_2-CH_2–CH_3), 0.90 (t, $J=7.33$, 3H, –CH_3). Anal. calcd ($C_{12}H_{15}NO_4S_2$): C, 47.82; H, 5.02; N, 4.65; S, 21.38. Found: C, 47.72; H, 5.13; N 4.58; S, 20.54; MS: 301, 284, 258, 229, 165.

4.1.15. *N*-benzyl-5-benzo[*b*]thiophenesulphonamide 1,1-dioxide (19). Starting from 5-chlorosulfonylbenzo[*b*]-thiophenesulphonamide 1,1-dioxide and benzylamine, compound **19** was obtained and recrystallised from isopropanol (71%): Mp 183.7 °C; (KBr, cm^{-1}): 1149, 1303 (SO_2), 3258 (NH). ^1H NMR (DMSO-d_6 δ): 8.51 (t, $J=6.23$, 1H, NH), 8.04 (d, $J_{67}=8.42$, 1H, H-6), 7.97–7.93 (m, 2H, H-7, H-4), 7.71 (d, $J_{32}=6.96$, 1H, H-3), 7.55 (d, 1H, H-2), 7.22 (s, 5H, –C_6H_5), 4.08 (d, 2H, –CH_2). Anal. calcd ($C_{15}H_{13}NO_4S_2$): C, 53.72; H, 3.91; N, 4.18; S, 19.12. Found: C, 53.71; H, 3.91; N, 4.16; S, 18.39.

4.1.16. *N*-(3-methoxy)phenyl-5-benzo[*b*]thiophenesulphonamide 1,1-dioxide (20). Starting from 5-chlorosulfonylbenzo[*b*]thiophenesulphonamide 1,1-dioxide and *m*-anisidine, compound **20** was obtained and recrcrystallized from water (28,3%): Mp 151 °C; (KBr, cm^{-1}): 1162, 1304 (SO_2), 1611 (OCH_3), 3248 (NH); ^1H NMR (DMSO-d_6 δ): 10.59 (s, 1H, NH), 8.1–7.9 (m, 3H, H-7, H-4), (d, $J_{67}=7.69$, 1H, H-6), 7.98 (d, 1H,), 7.92 (s, 1H, H-4), 7.76 (d, $J_{32}=6.69$, 1H, H-3), 7.54 (d, 1H, H-2), 7.14 (m, 1H, H-5′), 6.69–6.63 (m, 3H, H-2′, H-4′, H-6′), 3.67 (s, 3H, OCH_3). Anal. calcd ($C_{15}H_{13}NO_5S_2$): C, 51.27; H, 3.73; N, 3.99; S, 18.25. Found: C, 51.05; H, 3.65; N, 3.95; S, 18.73.

4.2. Biological methods

European Collection of Cell Cultures (ECACC) or American Type Culture Collection (ATCC) provided human tumour cell lines. Five cell lines were used: two human leukaemia (K-562 and CCRF-CEM) and three human solid tumours, one colon carcinoma (HT-29), one lung carcinoma (HTB54) and one melanoma (MEL-AC). MEL-AC cells were kindly provided by Dr. Natalia López-Moratalla (Universidad de Navarra, Pamplona, Spain). Human lung fibroblasts were kindly provided by Dr. Markus Nabholzs (ISREC, Epalinges, Switzerland). Cells were grown in RPMI 1640 medium (Life Technologies) supplemented with 10% foetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin and 10 mM HEPES buffer (pH = 7.4). The cytotoxic effect of each substance was tested at five different doses between 0.01 and 100 μM. Each substance was initially dissolved in DMSO at a concentration of 0.1 M, and serial dilutions were prepared using culture medium. The plates with cells from the different lines, to which medium containing the

substance under test were added, were incubated for 3 days at 37 °C in a humidified atmosphere containing 5% CO_2. Cytotoxicity was then determined by a colorimetric microassay based on the use of MTT.[12] Results are expressed as GI_{50}, concentration that reduced by 50% the growth of treated cells with respect to untreated controls.

Acknowledgements

R.V. is indebted to the Navarra Government for a grant.

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