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Apoptotic effects of thiazolobenzimidazole derivatives on sensitive and multidrug resistant leukaemic cells

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Abstract

We investigated the cytotoxic activity of eight thiazolobenzimidazole derivatives on sensitive HL60 and multidrug-resistant (MDR) (HL60R) leukaemia cell lines. The antitumour effects of these compounds were compared with those of RS-TBZ, a thiazolobenzimidazole derivative, previously described in our reports, that was able to induce apoptosis more markedly in MDR cells than in the parental sensitive cell lines. Only two compounds in this study proved to have interesting effects: (a) the S-enantiomer of TBZ, that was able to induce apoptosis in MDR cells in a slightly more selective manner than TBZ (racemic form); and (b) TBZ-4-OCH_3 (TBZ-4-OCH_3), that showed cytotoxic and apoptotic effects on sensitive and resistant leukaemia cells greater than TBZ, without cytotoxic effects on normal haemopoietic progenitor cells. Moreover, we observed that TBZ-4-OCH_3 was also active in cells expressing *Bcr-Abl*, an oncogene that confers resistance to apoptosis induced by several stimuli, including cytotoxic agents. The inhibition of caspase-9 and caspase-3 by specific polypeptide inhibitors decreased the apoptotic effects of TBZ-4-OCH_3 in HL60 cells indicating that apoptosis induced by this compound was, at least partly, caspase-mediated. On the contrary, the blocking of FL-associated cell surface antigen (Fas) using a specific Fas-blocking monoclonal antibody did not affect the level of apoptosis induced by TBZ-4-OCH_3 suggesting that the Fas pathway was not involved. In addition, the caspase 8 inhibitor was unable to inhibit the apoptotic activity of TBZ-4-OCH_3. The very low toxicity shown by TBZ-4-OCH_3 in normal haemopoietic progenitor cells and its high activity in sensitive and MDR neoplastic cells suggest a possible clinical use for this new compound.

Keywords: Leukaemia; Anticancer drugs; Thiazolobenzimidazole; Apoptosis; Multidrug resistance

1. Introduction

Recent studies demonstrated that a variety of widely used anticancer drugs kill cancer cells through the induction of apoptosis [1–4]. The molecular events leading to apoptosis involve a cascade of proteolytic cleavage events [5,6]. The most important proteins involved are the cysteine-dependent aspartate-directed proteases (caspases) which are initially expressed as inactive zymogens and are activated by proteolytic processing. Whereas the precise signals responsible for the induction of apoptotic cell death induced by anticancer drugs remain unclear, recent studies have demonstrated that cytochrome c released from the mitochondria contributes to this response [7]. Cytochrome c release into the cytosol results in apoptotic protease activating factor-1 (Apaf-1) activation and subsequent activation of caspases 9 and 3. The induction of apoptosis is associated with caspase-3-mediated cleavage of several substrates such as poly (ADP-ribose) polymerase (PARP) and protein kinase C sigma. In a similar manner, multiple caspases are activated during apoptosis triggered by FL-associated cell surface antigen (Fas) (cell determinant (CD)95/APO-1) ligation [8], and several authors observed that antineoplastic agents can induce apoptosis through a Fas/Fas-ligand-dependent pathway [9–11]. We

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have recently observed that the 2,6-difluorophenyl-thiazolobenzimidazole derivative called TBZ (RS-TBZ) was able to induce apoptosis selectively in the P-glycoprotein (Pgp)-expressing multidrug resistant (MDR) HL60R cell line [12]. This property was particularly interesting considering that in our study several classical antineoplastic agents were unable to induce apoptosis in HL60R cells when used at concentrations able to overcome the effects of Pgp. However, TBZ had a low cytotoxic activity: in fact it was active only at concentration of approximately 50 μM. Thus, in this work, we investigated the apoptotic activity in neoplastic cells of novel thiazolobenzimidazole derivatives in order to identify compounds more active than TBZ. The toxic effects on haemopoietic normal cells were also investigated.

2. Materials and methods

2.1. Cell culture

Continuous neoplastic cells (HL60, HL60R, K562 and K562ADR) were grown in Roswell Park Memorial Institute (RPMI) 1640 (Gibco Grand Island, NY, USA) containing 10% fetal calf serum (FCS) (Gibco), 100 U/ml penicillin (Gibco), 100 μg/ml streptomycin (Gibco), and 2 mM L-glutamine (Sigma Chemical Co., St Louis, MO, USA) in a 5% CO_2 atmosphere at 37°C. MDR HL60R and K562ADR cells were selected from parental sensitive HL60 and K562 cells by continuous exposure to increasing concentrations of daunorubicin (HL60R) or doxorubicin.

2.2. Chemicals

Daunorubicin, etoposide and mitoxantrone were purchased from Sigma Chemical Company. The caspase inhibitors Ac-DEVD-CHO (acetyl-Asp-Glu-Val-Asp-aldehyde), Ac-Z-LEHD-fmk (Z-Leu-Glu(Ome)-His-Asp(Ome)-fmk), Ac-IETD-CHO (N-acetyl-Ile-Glu-Thr-Asp-CHO) (aldehyde) and Z-VAD-fmk (benzyl-oxycarbonyl-Val-Ala-Asp-fluoromethyl ketone) were purchased from Alexis Biochemicals (Laufelfingen, Switzerland). The Fas agonistic antibody CH11 and the anti-Fas Ab ZB4 were purchased from Upstate Biotechnology (Lake Placid NY, USA). All other reagents were analytical grade.

2.3. Synthesis of thiazolobenzimidazole derivatives (TBZs)

The one-pot synthesis of 1H, 3H-thiazolo[3,4-a]benzimidazoles (TBZs) was carried out by a condensation–cyclisation reaction between o-phenylenediamine 1, aromatic aldehydes 2 and mercaptoacetic acid 3 in refluxing benzene (18a,b) as shown in Fig. 1.

In order to explore the influence of molecular modifications on biological activity, we synthesised derivatives with substituents on the C-1 phenyl ring having different electronic, steric and lipophilic properties. The compounds were obtained in good yields and characterised by spectroscopic methods.

2.4. Drug preparation

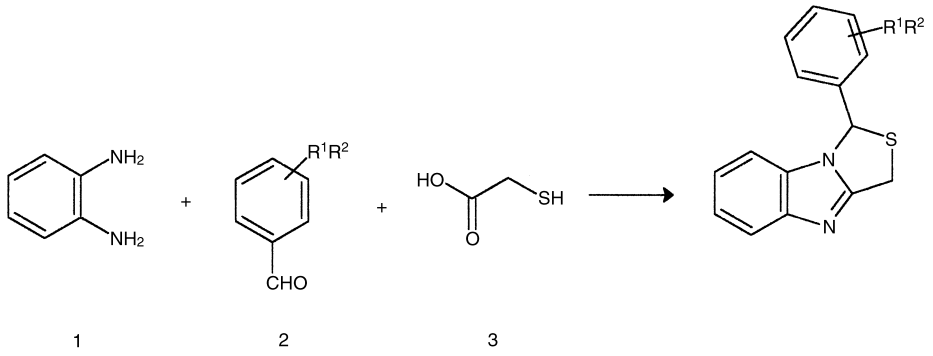
Each thiazolobenzoimidazole derivative was dissolved in dimethylsulphoxide (DMSO) in a stock solution at a concentration of 20 mM, stored at −20°C and protected from the light. In each experiment, DMSO never exceeded 0.5% and this percentage did not interfere with cell growth.

2.5. Cytotoxicity assays

To evaluate the number of live and dead neoplastic cells, the cells were stained with trypan blue and counted on a haemocytometer. To determine the growth inhibitory activity of the drugs tested, 2×10^5 cells were plated into 25 mm wells (Costar, Cambridge, UK) in 1 ml of complete medium and treated with different concentrations of each drug. After 48 h of incubation, the number of viable cells was determined and expressed as a per cent of control proliferation. To evaluate the cytotoxic effects on the haemopoietic progenitor cells, mononucleated cells obtained from bone marrow aspirates of five normal volunteers were treated with different concentrations of each drug. The *in vitro* clonal assays for haemopoietic progenitor cells were performed as described in Ref. [13]. Briefly, 3–5 ml bone marrow were diluted in RPMI 1640, layered over Ficoll-Hypaque gradients (density, 1.077), centrifuged at 400*g* for 30 min, and the interface mononuclear cells collected. The interface cells were washed three times in phosphate buffered saline (PBS), counted, and resuspended at a concentration of 1×10^5 in modified Eagle medium (MEM) containing 0.9% methylcellulose, 30% FCS, 10^{-5} M 2-mercaptoethanol, 5% phytohemoagglutinin lymphocyte culture medium (PHA–LCM), and 1 IU human erythropoietin in 15-mm plastic dishes. After 14 days of culture at 37°C in an environment of 5% CO_2 and 100% humidity, the number of colony forming unit (CFU)-multipotential (GEMM), CFU-granulocyte-macrophage (GM), and CFU-erythroid (E) was evaluated.

2.6. Flow cytometry analysis of the cell cycle and apoptosis

The cells were washed once in ice-cold PBS and resuspended at 1×10^6 ml in a hypotonic fluorochrome solution containing propidium iodide (Sigma) 50 μg/ml in 0.1% sodium citrate plus 0.03% (v/v) nonidet P-40 (Sigma). After a 30 min incubation, the samples were



	R^1	R^2
TBZ-H	H	H
TBZ-2,6-Cl_2	2-Cl	6-Cl
TBZ-2,3-$(OCH_3)_2$	2-OCH_3	3-OCH_3
TBZ-3-OCH_3	3-OCH_3	H
TBZ-2-F	2-F	H
RS-TBZ	2-F	6-F
S-TBZ	2-F	6-F
R-TBZ	2-F	6-F
TBZ-4-OCH_3	4-OCH_3	H

Fig. 1. Chemical structure of synthesised thiazolobenzimidazole derivatives.

filtered through Nylon cloth, and their fluorescence was analysed as single-parameter frequency histograms using a fluorescent activated cell sorter (FACScan) flow cytometer. Apoptosis was determined by evaluating the percentage of hypoploid nuclei accumulated in the sub-G_0G_1 peak after labelling with propidium iodide [13].

2.7. Morphological evaluation of apoptosis and necrosis

Drug-induced apoptosis and necrosis were determined morphologically after labelling with acridine orange and ethidium bromide [14]. The cells (2×10^5) were centrifuged (300*g*) and the pellet was resuspended in 25 μl of the dye mixture. Ten microlitres of the mixture was examined in oil immersion with a 100× objective using a fluorescence microscope. Live cells were determined by the uptake of acridine orange (green fluorescence) and exclusion of ethidium bromide (red fluorescence). Live and dead apoptotic cells were identified by perinuclear condensation of chromatin stained by acridine orange or ethidium bromide, respectively, and by the formation of apoptotic bodies. Necrotic cells were identified by uniform labelling of the cells with ethidium bromide.

3. Results

3.1. Cytotoxicity assay

The chemical structure of the new thiazolobenzoimidazole derivatives used in this study are shown in Fig. 1. The stability and the solubility in DMSO was examined and no difference was observed between the new derivatives and TBZ (RS-TBZ). Table 1 shows the cytotoxic activity, expressed as inhibitory concentration (IC) 50 and IC90, of these compounds in sensitive HL60 and MDR HL60R cells. TBZ-4-OCH_3 (TBZ-4-OMe), characterised by the presence of a methoxy group at the 4-position of the C-1 phenyl substituent, was the most active compound in both the sensitive and resistant cell lines. Compounds TBZ-2,3-$(OCH_3)_2$ and TBZ-2,6-Cl_2 showed a slightly higher cytotoxic activity in HL60 cells compared with RS-TBZ, but were less effective in the resistant HL60R cells. The R-enantiomer of TBZ was more active than RS-TBZ in both the sensitive and resistant cells, while the S-enantiomer was more active in the HL60R cell line but less active in the sensitive cells.

3.2. Effects of TBZ derivatives on normal haematopoietic precursors

To evaluate the myelotoxicity induced by TBZ derivatives, we studied the effects of RS-TBZ, R-TBZ, S-TBZ and TBZ-4-OCH_3 on the growth of human haemopoietic progenitors CFU-GEMM, CFU-GM and CFU-E by clonal assay (Fig. 2). At the concentrations active on HL60 and HL60R cells, the racemic TBZ and the R- and S- enantiomers did not show any toxicity on CFU-GEMM and CFU-GM, while, a considerable toxicity was observed on erythroid precursors when these drugs were used at concentrations greater than 75 μM. Interestingly, TBZ-4-OCH_3 did not show any inhibition of myeloid and erythroid precursors when used at concentrations greater than those active in HL60 or HL60R cells (25 μM).

3.3. Apoptosis and cell cycle

Fig. 3(a) and (b) shows the apoptotic effects of RS- S- and R-TBZ used at concentrations of 50 μM, 75 μM and 100 μM in HL60 and HL60R cells. At all concentrations used, S-TBZ was the less active in HL60 cells, but it showed an apoptosis-inducing activity similar to RS- and R-TBZ in the MDR HL60R cells.

TBZ-4-OCH_3 was the thiazolobenzoimidazole derivative that was more active in inducing apoptosis in HL60 and HL60R cell lines. It was approximately 24-fold more active than R-TBZ in HL60 cells and approximately 5-fold more active than RS-, S and R-TBZ in the HL60R cells, respectively (Fig. 3c). Flow cytometry assay shows an evident apoptotic pre-G_0G_1-peak with a decrease of cells in the G_0G_1-phase in the HL60 and HL60R cells treated with 5 or 10 μM TBZ-4-

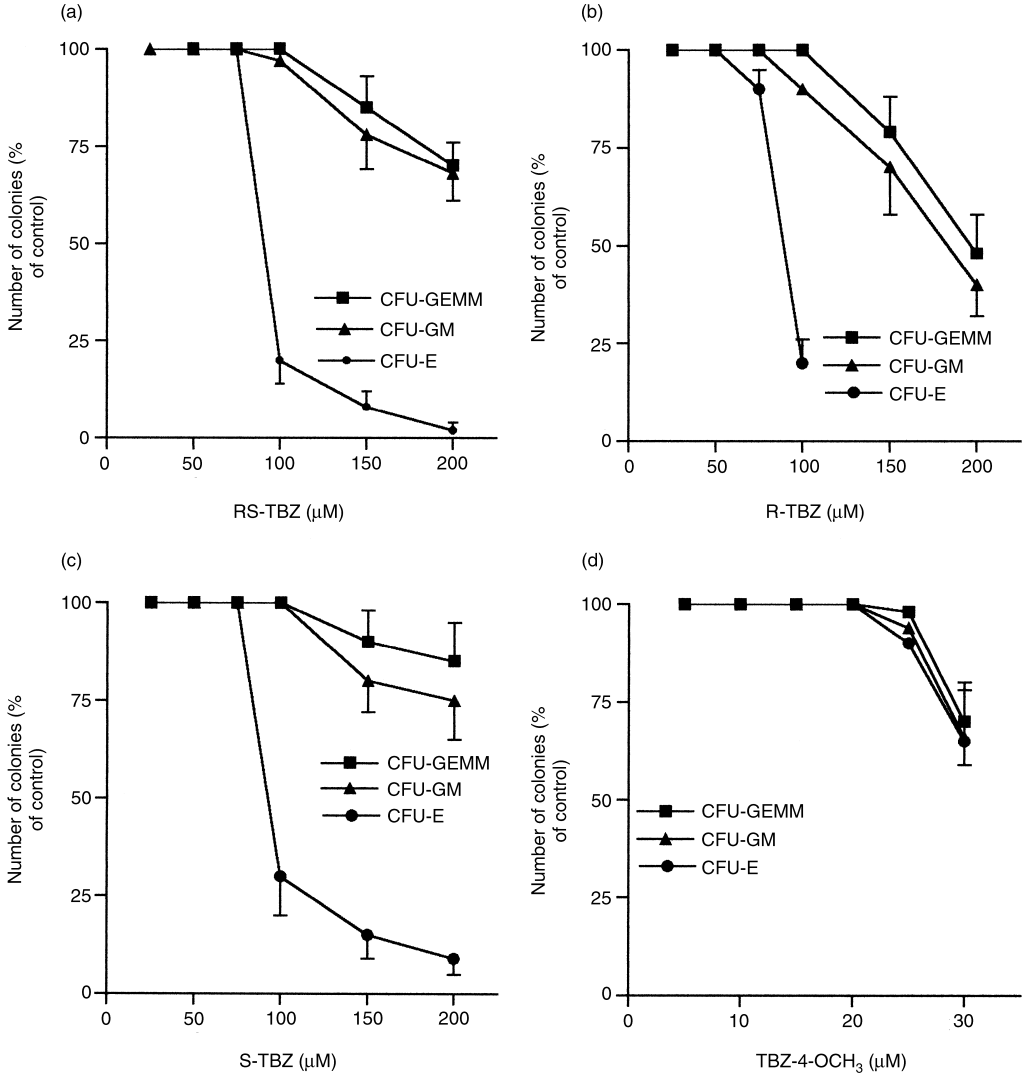


Fig. 2. Effects of (a) RS-TBZ; (b) R-TBZ; (c) S-TBZ and (d) TBZ-4-OCH_3 on colony forming unit (CFU) GEMM, CFU-GM and CFU-E. Results are means ±standard deviation (S.D.) of five experiments performed on mononucleated cells obtained from bone marrow aspirates of five normal volunteers. CFU-GEMM, colony forming unit-multipotential; CFU-GM, colony forming unit-granulocyte macrophage; CFU-E, colony forming unit-erythroid.

OCH_3 (Fig. 4). Interestingly, TBZ-4-OCH_3 was also able to induce apoptosis in the K562 cells, a fusion product of the bcr and c-Abelson genes (Bcr-Abl)-expressing cell line that is resistant to apoptosis caused by several cytotoxic agents. As shown in Table 2, chemotherapeutic drugs commonly used in the treatment of leukaemia, such as daunorubicin, etoposide and mitoxantrone, were able to induce apoptosis in K562 cells only at concentrations many times greater than those effective in the HL60 cells. On the contrary, the activity of TBZ-4-OCH_3 in the K562 cells and in the MDR counterpart K562ADR was similar to that observed in HL60 and HL60R cells (Fig. 3c and d). These data indicate that the apoptosis-inducing activity of TBZ-4-OCH_3 is unaffected by the presence of the *Bcr-Abl* oncogene in the neoplastic target cells. Moreover, we examined whether TBZ-4-OCH_3 was able to reverse the MDR in HL60R and K562ADR cells. We treated these MDR cell lines with different subtoxic concentrations of TBZ-4-OCH_3 (0.1, 0.5 or 1 μM) and, after 2 h, with daunorubicin (0.1 μg/ml for HL60R and 0.25 μg/ml for K562ADR). TBZ-4-OCH_3 did not modify the resistance (in terms of cytotoxicity and apoptosis) to daunorubicin. On the contrary, verapamil used at a concentration of 5 μg/ml, or cyclosporin A (2 μg/ml) were able to sensitise MDR cells to the cytotoxic effects of daunorubicin (data not shown).

3.4. *Effects of Fas and caspase inhibitors on the apoptosis induced by TBZ-4-OCH_3*

Previously, we observed that HL60 is a cell line that expresses the cell death receptor Fas and these cells undergo apoptosis after treatment with the agonistic

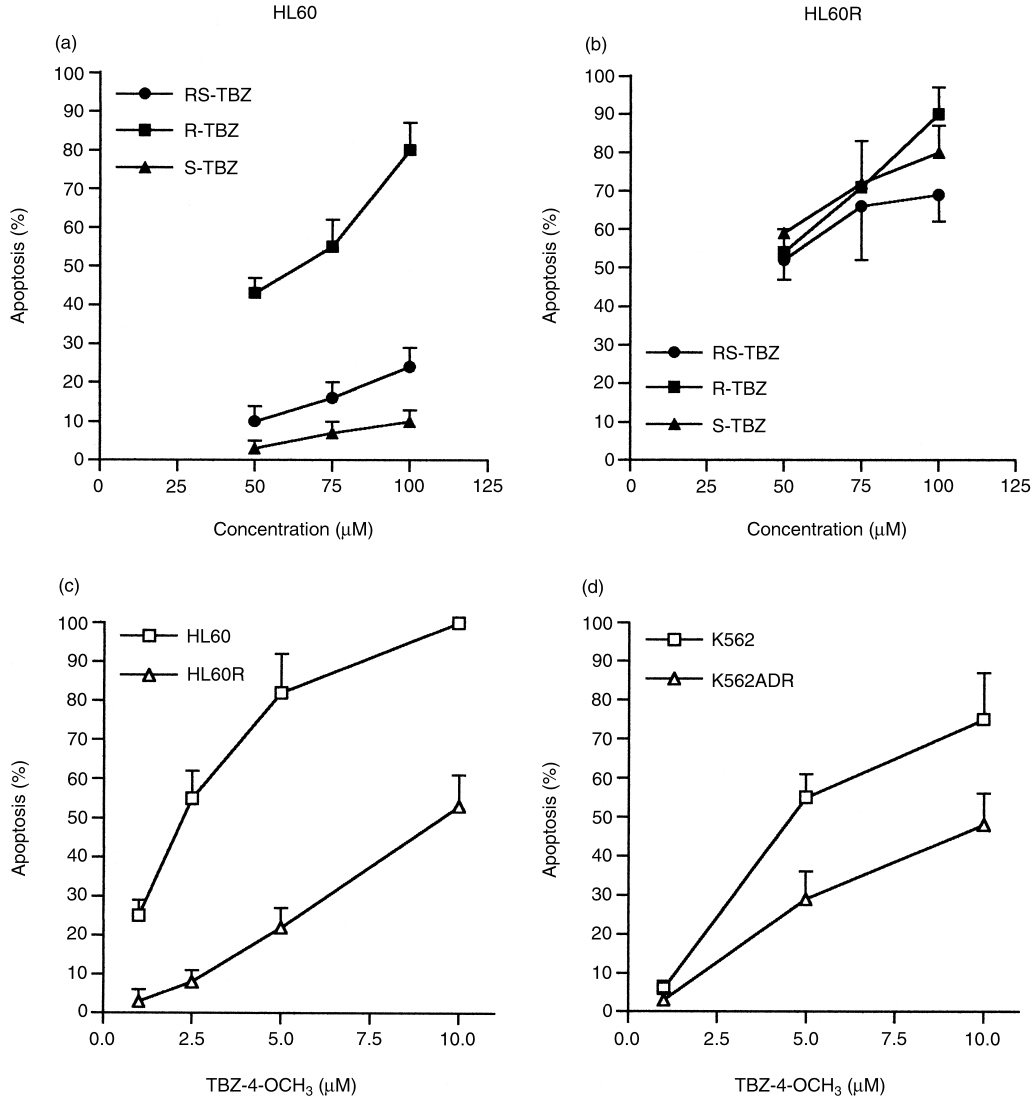


Fig. 3. Apoptosis-inducing activity, evaluated by morphological examination of RS-TBZ, R-TBZ, S-TBZ and TBZ-4-OCH_3 in HL60 and HL60R cell lines (a, b and c). (d) Apoptosis-inducing activity of TBZ-4-OCH_3 in K562 and K562ADR cells. Results are means ±standard deviations (S.D.) of at least five experiments.

anti-Fas monoclonal antibody CH11 [15]. To understand if TBZ-4-OCH_3-induced apoptosis was Fas-mediated, we treated HL60 cells with a Fas-blocking monoclonal antibody (ZB4) or with the caspase 8 inhibitor Ac-IETD-CHO, and, after 2 h with 2.5 μM TBZ-4-OCH_3. As shown in Fig. 5, these inhibitors were unable to inhibit TBZ-4-OCH_3-induced apoptosis which was, on the contrary, partially inhibited by the pan-caspase inhibitor Ac-Z-VAD-fmk, by the caspase 9 inhibitor Ac-Z-LHED-fmk and by the caspase 3 inhibitor Ac-DEVD-CHO.

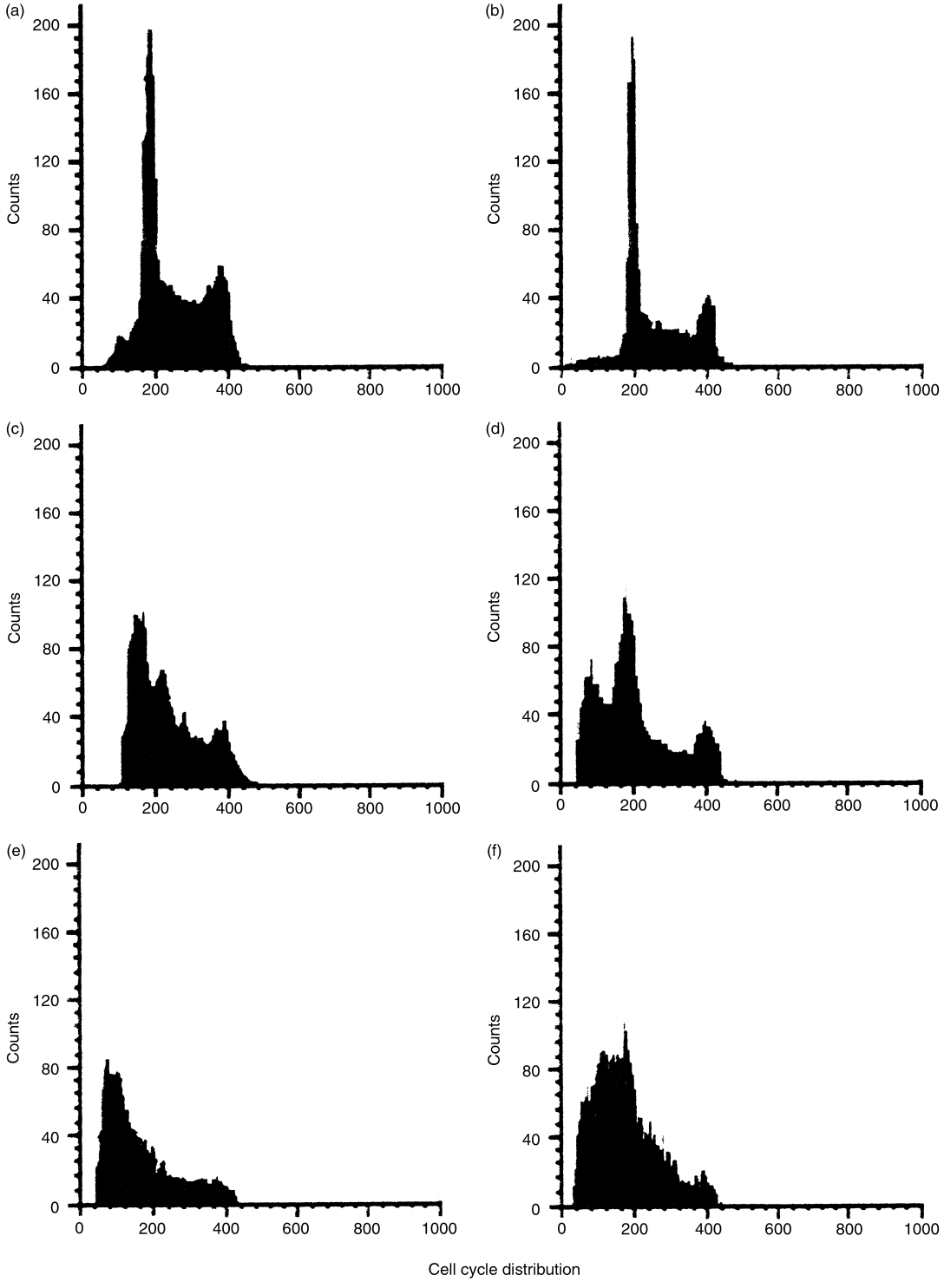


Fig. 4. Flow cytometric assay of cell cycle distribution of HL60 and HL60R cells after 48 h exposure to TBZ-4-OCH_3. (a) Untreated HL60 cells; (b) untreated HL60R cells; (c) HL60 cells treated with 5 μM TBZ-4-OCH_3; (d) HL60R cells treated with 5 μM TBZ-4-OCH_3; (e) HL60 cells treated with 10 μM TBZ-4-OCH_3 and (f) HL60R cells treated with 10 μM TBZ-4-OCH_3.

Table 1
IC50 and IC90 of different thiazolobenzimidazole derivatives in sensitive HL60 and in multidrug resistant HL60R cell lines

	HL60		HL60R	
	IC50 (μM)	IC90 (μM)	IC50 (μM)	IC90 (μM)
TBZ-H	75 (±8)	180 (±13)	120 (±18)	210 (±11)
TBZ-2,6-Cl_2	25 (±4)	100 (±10)	100 (±12)	250 (±25)
TBZ-2,3-$(OCH_3)_2$	23 (±3)	100 (±9)	120 (±20)	280 (±23)
TBZ-3-OCH_3	40 (±16)	240 (±17)	75 (±8)	270 (±11)
TBZ-2-F	80 (±6)	250 (±17)	130 (±16)	300 (±22)
RS-TBZ	50 (±9)	150 (±13)	55 (±5)	100 (±7)
S-TBZ	75 (±10)	250 (±22)	25 (±3)	75 (±9)
R-TBZ	25 (±4)	85 (±12)	20 (±3)	60 (±8)
TBZ-4-OCH_3	1 (±0.2)	5 (±0.2)	3 (±0.4)	10 (±2)

Evaluation after 48 h of treatment. Results are means ±S.D. of at least five experiments.

IC50, inhibitory concentration required for 50% inhibition; IC90, inhibitory concentration required for 90% inhibition; S.D., standard deviation.

Table 2
Apoptosis inducing activity evaluated as AC70 (drug concentration able to induce 70% apoptosis) of TBZ-4-OCH_3 and other antitumoral drugs in HL60 and K562 cell lines

	AC70 (μM)		
	HL60	K562	R[a]
Daunorubicin	0.028 (±0.005)	0.56 (±0.1)	20
Etoposide	0.1 (±0.02)	100 (±20)	1000
Mitoxantrone	0.005 (±0.001)	0.5 (±0.09)	100
TBZ-4-OCH_3	3.8 (±0.7)	8 (±1.6)	2.1

Evaluation after 48 h of treatment. Results are means ±S.D. of at least five experiments. S.D., standard deviation.

[a] Ratio between AC70 in K562 cells and AC70 in HL60 cells.

4. Discussion

TBZ is an anti-HIV thiazolobenzoimidazole derivative able to inhibit the HIV-1 reverse transcriptase [16–19]. We had previously observed that TBZ was active as an antitumoral agent both in sensitive and MDR cell lines when used at concentrations higher (75–100 μM) than those required to exert its antiviral activity (1 μM). Furthermore, TBZ has been shown to inhibit the growth of leukaemic HL60 cells with a recruitment in the G_0G_1 phase of the cell cycle. Moreover, it induced a more marked apoptosis in MDR HL60R cells compared with the sensitive parental HL60 cell line [12]. In this study, the antitumour activity of novel thiazolobenzoimidazole derivatives was investigated, which were synthesised with the aim of identifying more active TBZ analogues. The compounds TBZ-2,6-Cl_2 and TBZ-2,3-$(OCH_3)_2$, were more active than RS-TBZ in HL60-sensitive cells, but were less effective in the MDR HL60R cells. The R and S enantiomers of TBZ showed a similar cytotoxicity in the HL60R cells, while, in the sensitive HL60 cells, the activity of these enantiomers was markedly different, especially in terms of the induction of apoptosis. In fact, the S-enantiomer completely failed to induce apoptosis when used at a concentration of 100 μM. In contrast, the R-enantiomer exhibited a similar apoptotic activity in the HL60 cells to that observed in the HL60R cells.These data indicate

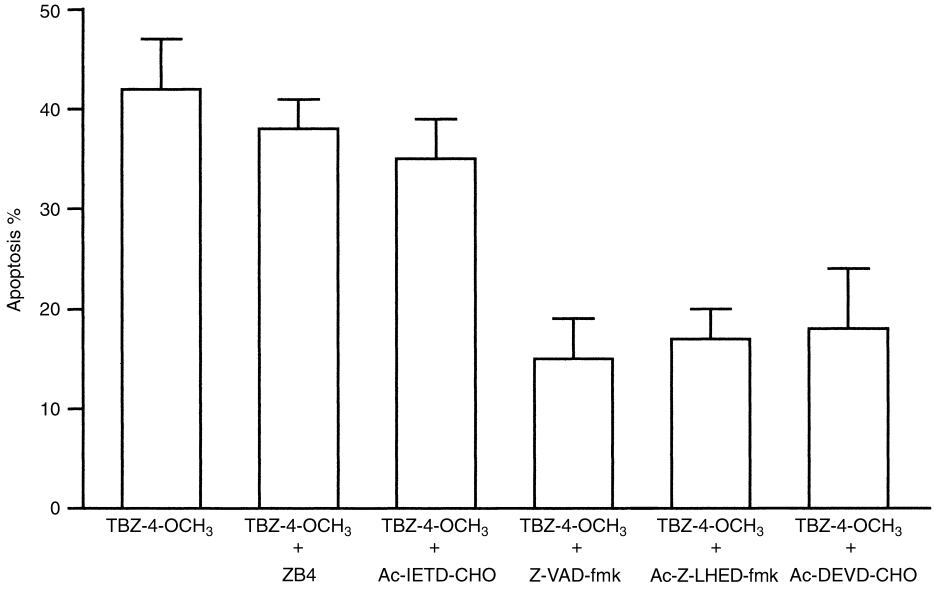


Fig. 5. Effects of the Fas-blocking monoclonal antibody ZB4, the caspase-8 inhibitor Ac-IETD-CHO, the pan-caspase inhibitor Z-VAD-fmk, the caspase-9 inhibitor Ac-Z-LEHD-fmk, and the caspase-3 inhibitor Ac-DEVD-CHO on the apoptotic-inducing activity of TBZ-4-OCH_3 in HL60 cells. Cells were incubated with 200 μM of each caspase inhibitor or with 0.5 μg/ml of ZB4 and, after 2 h, with 5 μM TBZ-4-OCH_3. Evaluation was after 24 h of treatment. Results are means ±standard deviation (S.D.) of at least five experiments.

that the selective apoptosis-inducing activity of RS-TBZ in MDR cells depends only on the presence of the S-component of the racemic compound.

The derivative TBZ-4-OCH_3 was the most active compound identified in this study. It showed an IC50 25 times lower than TBZ-2,6-Cl_2 and 23 times lower than TBZ-2,3-$(OCH_3)_2$, in HL60 cells and approximately 7 and 8 times lower than the R- and S-TBZ derivatives, respectively, in the HL60R cells. TBZ-4-OCH_3 induced cytotoxicity in HL60 cells at concentrations comparable with those required for TBZ to exert its antiviral activity (1 μM). Moreover, TBZ-4-OCH_3 did not show any toxicity in normal haemopoietic progenitor cells, even when used at high concentrations (25 μM). We also observed that TBZ-4-OCH_3 exerts its antitumour activity by primarily inducing programmed cell death in the target cells. This activity is, at least in part, caspase-mediated, as shown by the ability of caspase inhibitors to reduce the percentage of apoptotic cells. In particular, caspase 9 and caspase 3 seem to be involved in the TBZ-4-OCH_3-induced apoptosis. On the contrary, apoptosis was not modified by the inhibitor of caspase 8. Moreover, the use of a Fas receptor blocking monoclonal antibody did not inhibit apoptosis, confirming that the Fas pathway is not involved in the programmed cell death induced by TBZ-4-OCH_3. Another important property of this compound was its activity in cells expressing the Philadelphia chromosome such as the K562 cell line. The Philadelphia chromosome is the product of a reciprocal exchange between the long arms of chromosomes 9 and 22. This fusion event results in a hybrid gene in which the amino terminal sequence of the *bcr* gene on chromosome 22 is fused to the second exon of the *c-abl* gene on chromosome 9 [20]. The resulting Bcr–Abl protein product is an oncoprotein that confers resistance to apoptosis induced by many anticancer drugs such as etoposide, actinomycin D, cycloheximide and dexamethasone [3,21–24]. We observed that TBZ-4-OCH_3 induced apoptosis in K562 cells when used at a concentration only 2.1 higher than the concentration effective in HL60 cells. These data are significant considering that drugs commonly used in the treatment of leukaemia such as daunorubicin, etoposide and mitoxantrone are active in terms of apoptosis, in K562 cells at concentrations many times higher than the concentrations that are active in HL60 cells.

In conclusion, two interesting thiazolobenzoimidazole derivatives have been identified: the S-TBZ, characterised by a selective action in MDR cells greater than that previously described for the racemic TBZ, and the TBZ-4-OCH_3 that showed a potent antitumour activity in sensitive and MDR leukaemia cells with very low toxic effects in normal haemopoietic progenitor cells. Like TBZ, these thiazolobenzoimidazole derivatives exert their antitumour effects by activating the programmed cell death pathway. At the moment, the mechanisms of the selective activity in MDR cells shown by S-TBZ are not known; however, this property may be useful in the treatment of MDR malignancies to selectively kill only Pgp-expressing neoplastic cells while preserving Pgp-negative normal cells.

References

1. Collins RJ, Harmon BV, Souvlis T, Pope JH, Kerr JFR. Effects of cycloheximide on B-chronic lymphocytic leukaemic and normal lymphocytes in vitro: induction of apoptosis. *Br J Cancer* 1991, **64**, 518–522.
2. Walker PR, Smith C, Youdale T, Leblanc J, Whitfield JF, Sikorska M. Topoisomerase II-reactive drugs induce apoptosis in thymocytes. *Cancer Res* 1991, **51**, 1078–1084.
3. Martins LM, Mesner PW, Kottke TJ, *et al*. Comparison of caspase activation and subcellular localization in HL-60 and K562 cells undergoing etoposide-induced apoptosis. *Blood* 1997, **11**, 4283–4296.
4. Vial JP, Belloc F, Dumain P, *et al*. Study of the apoptosis induced in vitro by antitumoral drugs on leukaemic cells. *Leukemia Res* 1997, **21**, 163–172.
5. Salvesen GS, Dixit VM. Caspases: intracellular signaling by proteolysis. *Cell* 1997, **91**, 443–446.
6. Alnemri ES. Mammalian cell death proteases: a family og highly conserved aspartate specific proteases. *J Cell Biochem* 1997, **64**, 33–42.
7. Decaudin D, Geley S, Hirsch T, *et al*. Bcl-2 and Bcl-xL antagonize the mitochondrial dysfunction preceding nuclear apoptosis induced by chemotherapeutic agents. *Cancer Res* 1997, **57**, 62–67.
8. Nagata S, Golstein P. The Fas death factor. *Science* 1995, **267**, 1449–1455.
9. Friesen C, Herr I, Krammer PH, Debatin K-M. Involvement of the CD95 (APO-1/Fas) receptor/ligand system in drug-induced apoptosis in leukemia cells. *Nature Med* 1996, **5**, 574–577.
10. Muller M, Strand S, Hug H, *et al*. Drug-induced apoptosis in hepatoma cells is mediated by the CD95 (APO-/Fas) receptor/ligand system and involves activation of wild-type p53. *J Clin Invest* 1997, **99**, 403–413.
11. Fulda S, Sieverts H, Friesen C, Herr I, Debatin KM. The CD95 (APO-/Fas) system mediates drug-induced apoptosis in neuroblastoma cells. *Cancer Res* 1997, **57**, 3823–3829.
12. Grimaudo S, Tolomeo M, Chimirri A, Zappala M, Gancitano RA, D'Alessandro N. Selective induction of apoptosis in multidrug resistant HL60R cells by the thiazolobenzoimidazole derivative 1-(2,6-difluorophenyl)-1H,3H-thiazolo 3,4-a(benzimidazole) (TBZ). *Eur J Cancer* 1998, **11A**, 1756–1763.
13. Darziynkiewick Z, Bruno S, Del Bino G, *et al*. Features of apoptotic cells measured by flow cytometry. *Cytometry* 1992, **13**, 795–798.
14. Duke RC, Cohen JJ. Morphological and biochemical assays of apoptosis. In Coligan JE, Kruisbeak AM, *et al*., eds. *Current Protocols in Immunology*. New York, John Wiley & Sons, 1992, 3.17.1.
15. Tolomeo M, Grimaudo S, Cannizzo G, *et al*. Implication of Fas/APO-1 and NF-kB in resistance to drug-induced apoptosis in MDR leukemic cells. *Haematologica* 1999, **84**, 291.
16. Hizi A, Tal R, Shaharabany M, *et al*. Specific inhibition of the reverse transcriptase of human immunodeficiency virus type 1 and the chimeric enzymes of human immunodeficiency virus type 1 and type 2 nonnucleoside inhibitors. *Antimicrob Agents Chemother* 1993, **37**, 1037–1042.
17. Artico M. Non nucleoside anti-HIV reverse transcriptase inhibitors (NNRTIs); a chemical survey from lead compounds to select drugs for clinical trials. *Il Farmaco* 1996, **51**, 305–331.
18. Chimirri A, Grasso S, Molica C, *et al*. Structural features and anti-HIV activity of the isomers of 1-(2′,6′-difluorophenyl)-

1H,3H-thiazolo(3,4-a)benzimidazole, a potent non-nucleoside HIV-1 reverse transcriptase inhibitor. *Antiviral Chem Chemother* 1997, **8**, 83–90.

19. Chimirri A, Grasso S, Monforte AM, Monforte P, Zappala' M. Anti-HIV agents II: synthesis and in vitro anti-HIV activity of novel 1H,3H-thiazolo(3,4-a)benzimidazoles. *Il Farmaco* 1991, **46**, 925–933.
20. Groeffen J, Heistenkamp N. The BCR/ABL hybrid gene. In Goldman JM, ed. *Chronic Myeloid Leukemia*. London, Bailliere Tindall, 1987, 983–999.
21. Bedi A, Zehnbauer A, Barber J, Sharkis S, Jones R. Inhibition of apoptosis by BCR-ABL in chronic myeloid leukemia. *Blood* 1994, **83**, 2038–2040.
22. McGahon A, Bissonette RP, Schmitt M, Cotter K, Green DR, Cotter TG. Bcr-Abl maintains resistance of chronic myelogenous leukemia cells to apoptotic cell death. *Blood* 1994, **83**, 1179–1187.
23. McGahon JA, Brown DG, Martin SJ, *et al*. Downregulation of Bcr-Abl in K562 cells restores susceptibility to apoptosis: characterization of the apoptotic death. *Cell Death Different* 1997, **4**, 95–104.
24. Bedi A, Barber JP, Bedi GC, *et al*. BCR–ABL-mediated inhibition of apoptosis with deley of G2/M transition after DNA damage: a mechanism of resistance to multiple anticancer agents. *Blood* 1995, **86**, 1148–1158.