Cytotoxic Effects of *N*'-Formyl-2-(5-nitrothiophen-2-yl) benzothiazole-6-carbohydrazide in Human Breast Tumor Cells by Induction of Oxidative Stress

JUAN R. RODRIGUES[1], JAIME CHARRIS[2], JOSÉ CAMACHO[2], ARTHUR BARAZARTE[2], NEIRA GAMBOA[2] and FERNANDO ANTUNES[1]

[1]*Laboratory of Biochemistry of Oxidants and Antioxidants, Centre and Department of Chemistry and Biochemistry, School of Sciences, University of Lisbon, Lisbon, Portugal;*
[2]*Laboratory of Organic Synthesis and Biochemistry, School of Pharmacy, Central University of Venezuela, Los Chaguaramos, Caracas, Venezuela*

Abstract. *Chemically active molecules, such as reactive oxygen species (ROS), are prone to induce cellular damage by oxidative stress and this could be exploited as a strategy to kill malignant cells. In this study, we evaluated the antitumor activity of a new compound, N'-formyl-2-(5-nitrothiophen-2-yl)benzothiazole-6-carbohydrazide (FBZC) by assessing its pro-oxidant effects on breast cancer in vitro. Oxidative stress, generated by FBZC, was characterized by measuring reactive species and antioxidant enzymes and markers. Results showed that the cytotoxic effects of FBZC on MCF7 breast cancer cells (half inhibitory concentration of 5.4 μg/ml), were partially reversed by the addition of regular antioxidants. FBZC induced ROS and lipid peroxidation, together with a significant inhibition of superoxide dismutase, glutathione reductase and total glutathione levels as well as increases in catalase and glutathione-S-transferase activities, in an acute fast response. Thus, the antitumor effects of FBZC could be related to oxidative deregulation due to a combination of induction of ROS generation and inhibition of key antioxidant enzymes.*

Breast cancer represents the most commonly diagnosed malignancy, after skin cancer, and the second leading cause of cancer-related death among women (1). New alternative therapies for this disease have emerged, however, most of them are associated with significant side-effects, high economic costs and reduced quality of life (2); thus the development of novel alternatives for this malignancy is a major priority.

Benzothiazole compounds have demonstrated highly selective antitumor activities *in vitro* (3, 4). Several such derivatives have been synthesized as pro-drugs and have already been introduced in clinical trials (5). On the other hand, nitrothiophene compounds are also known as potential cytotoxics, representing promising new leading analogs against cancer, possibly by inducing DNA damage (6). Both chemical structures have shown antitumor activities by inducing generation of reactive oxygen species (ROS) and apoptosis, involving nucleophilic attack by intracellular thiols (7, 8).

ROS are molecules characterized by their chemically active and unstable properties, being able to induce cellular damage. In this context, they could be useful due to their ability to kill tumor cells, which are susceptible to oxidative stress (9). Thus, we evaluated the potential antitumor activity of a new compound, *N*'-formyl-2-(5-nitrothiophen-2-yl)benzothiazole-6-carbohydrazide (FBZC), which combines a benzothiazole and a 5-nitrothiophene in the same molecule, in a human breast cancer cell line by induction of oxidative stress and by the ensuing antioxidant response.

Materials and Methods

Synthesis of FBZC. The synthesis and physicochemical evaluations of FBZC (Figure 1) were performed according to Charris *et al*. (10). The melting point was determined on a Thomas micro hot stage apparatus and infrared spectra as KBr pellets on a Shimadzu model 470 spectrophotometer. The ^{1}H-nuclear magnetic resonance, spectrum was recorded using a Jeol Eclipse 270 (270 MHz) spectrometer using dimethyl sulfoxide (DMSO)-d_6, and was reported in ppm downfield from the residual DMSO. Elemental analyses were performed on a Perkin Elmer 2400 CHN analyzer, results were within ± 0.4% of the predicted values for all

Correspondence to: Juan Rodrigues, Departamento de Química e Bioquímica, Edifício C8, piso 4, sala 8.4.57. Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal. Tel: +351 217500916, Fax: +351 217500088, e-mail: juanricardorodrigues@gmail.com

Key Words: Oxidative stress, benzothiazole, glutathione reductase, antitumor, antioxidants, MCF7 breast cancer cells.

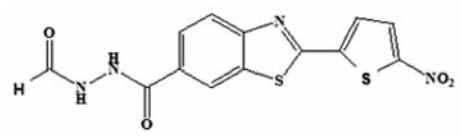

Figure 1. *Chemical structure of N'-formyl-2-(5-nitrothiophen-2-yl) benzothiazole-6-carbohydrazide (FBZC).*

compounds. Chemical reagents were obtained from Aldrich Chemical Co, St. Louis, Mo, USA. All solvents were distilled and dried in the usual manner.

Cell cultures. Breast adenocarcinoma cells (MCF7) and non-tumor retinal pigment epithelium cells (RPE) were obtained from the European Collection of Cell Cultures, Salisbury Wiltshire, UK. Cells were grown in RPMI medium supplemented with 10% fetal bovine serum (FBS), L-glutamine 1%, penicillin (50 units/mL) and streptomycin (50 μg/mL), in a humidified atmosphere of 95% air and 5% CO_2 at 37˚C. Cell culture media were from HyClone Laboratories, Inc. South Logan, UT, USA.

Time and dose-effects of FBZC on cell viability. A total of 5×10^3 MCF7 or 5×10^3 RPE cells were cultured in a 96-well microtiter plate containing 0.1 ml of RPMI growth media/well for 24 h. Cells were incubated with the compounds dissolved in dimethylsulfoxide (DMSO)/medium 0.02% (5-100 μg/mL, 72 h) and were evaluated for their cytotoxicity (11). The half inhibitory concentration (IC_{50}) values obtained were defined as the concentration of tested compounds resulting in a 50% reduction of the cell viability compared to vehicle-treated cells. Further evaluations of FBZC were performed using its IC_{50} value.

Effect of antioxidants on cytotoxicity of FBZC. A total of 5×10^3 MCF7 cells cultured for 24 hours in a 96-well microtiter plate containing 0.1 mL of RPMI growth medium/well were incubated with vehicle, FBZC IC_{50}, *N*-acetyl-cysteine (0.5 mM), or TROLOX (0.1 mM) in different incubation combinations (37˚C for 72 h). Cells were then incubated with Alamar Blue (37˚C for 4 h) and resorufin was measured at 570/600 nm. The results are expressed as the percentage of cell viability compared to that of the vehicle control.

Intracellular ROS levels. The determination of intracellular ROS after FBZC incubations over time was measured with the non-fluorescent probe 2',7'-dichlorofluorescein diacetate (DCFH-DA), as previously described (12).

Sample preparation. For the determination of lipid peroxidation, enzyme activities and total glutathione, 2×10^6 MCF7 cells were treated either with vehicle or FBZC at IC_{50} (37˚C for 3, 6, 12 and 24 h), cells were trypsinized and then 1×10^6 they were suspended in PBS and centrifuged (1200 ×*g* for 5 min). Samples were subjected to different protocols, depending to the enzyme activity/metabolite determinations.

Lipid peroxidation assay. To determine lipid peroxidation by the compound, we followed the procedure previously described (13), measuring the production of thiobarbituric reactive oxygen species (TBARs) at 535/580 nm.

Table I. *Cytotoxic effect of N'-formyl-2-(5-nitrothiophen-2-yl) benzothiazole-6-carbohydrazide (FBZC) compared with cisplatin and doxorubicin. Results are expressed as the mean±SEM of the IC_{50} values in four independent experiments.*

	IC_{50} (μg/mL)		Specificity
Compound	MCF7	RPE	(RPE/MCF7)
FBZC	5.4±1.7***†	113.8±5.71	21.07
Cisplatin	7.19±0.47	7.6±0.01	1.06
Doxorubicin	62.11±3.5	5.18±0.08	0.08

IC_{50}: Half inhibitory concentration; ***$p<0.001$ compared to doxorubicin. †$p<0.05$ compared to results for RPE cells.

Activities of superoxide dismutase (SOD) and catalase. SOD activity was measured according to McCord and Fridovich (14), which tracks the reduction of cytochrome c at 550 nm. The catalase activity was measured according to Aebi (15), which tracks the reduction of H_2O_2 at 240 nm.

Determination of glutathione level, glutathione reductase (GR) and glutathione-S-transferase (GST) activities. To determine the total glutathione level, we followed the procedure previously described by Tietze (16). The absorbance at 412 nm was registered at every 20 s for 5 min due to the formation of the reduced product, thionitrobenzene. To determine GR activity we followed a procedure previously described (17) and the decrease of the NADPH levels, at 340 nm, was recorded. For the GST activity, we followed the procedure previously described by Rice *et al*. (18), measuring the accumulation of *S*-(2,4-dinitrobenzyl) glutathione at 340 nm.

Determination of dehydrogenase enzyme activities. To determine the activities of glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH), we followed a procedure described by Tian *et al*. (19), measuring the fluorescence produced by the conversion of $NADP^+$ to NADPH + H^+ by either G6PDH or 6PGDH.

Protein levels of samples were determined according to Bradford (20). Data are presented as the average of four independent experiments (n=4) and tested for statistical significance using unpaired *t*-tests for specific group comparisons, assuming 95% confidence limits, using the GraphPad Prism 4.02 software for Windows, San Diego, CA, USA.

Results

FBZC (Figure 1) was synthesized and evaluated for its potential antineoplastic activity *in vitro*.

The ability of FBZC to induce cytotoxic effects on the human breast tumor cell line MCF7 is presented in Table I. FBZC was more than 20-fold more specific to the tumor cell line than to non-tumor cells. The results also highlighted the more toxic and specific effect of this compound compared to

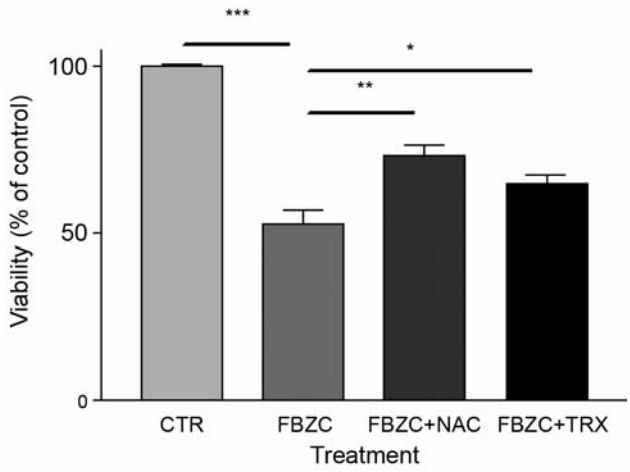

Figure 2. *Effects of antioxidants on the cytotoxicity of N'-formyl-2-(5-nitrothiophen-2-yl)benzothiazole-6-carbohydrazide (FBZC) on breast cancer MCF7 cells. FBZC was applied at its half inhibitory concentration (IC_{50}=5.4 μg/mL). Results are expressed as the mean±SEM. CTR: Vehicle-treated control; NAC: N-Acetyl-cysteine (0.5 mM); TRX: Trolox (0.1 mM); *p<0.05, **p<0.01 and ***p<0.001.*

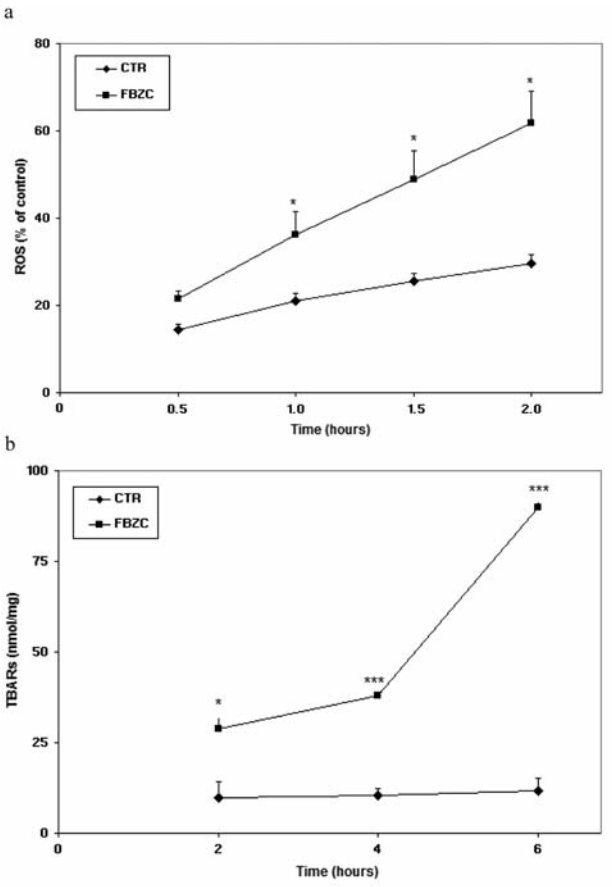

Figure 3. *Effects of N'-formyl-2-(5-nitrothiophen-2-yl)benzothiazole-6-carbohydrazide (FBZC) (5.4 μg/mL) on oxidative markers over time in breast cancer MCF7 cells. a: Levels of reactive oxygen species (ROS); b: levels of thiobarbituric acid-reactive species (TBARs). Results are expressed as the mean±SEM. *p<0.05 and ***p<0.001 compared to vehicle control (CTR).*

those of regular antineoplastics cisplatin and doxorubicin. The toxic effects on MCF7 cells were partially reversed by the presence of two functionally unrelated antioxidants suggesting that oxidative stress might be responsible for the cytotoxicity of FBZC (Figure 2).

FBZC increased the production of ROS, and lipid peroxidation, by TBARs, rose in a time-dependent manner and, consequently induced alteration in the normal oxidative status of cells (Figure 3).

Cells are well-equipped with a complete and integrated antioxidant defence system. SOD and catalase represent the first line of defence against ROS. FBZC inhibited SOD activity by approximately 25% and induced a non-monotonous effect on catalase due to a strong stimulatory response observed at the first 12 h of incubation; but its activity faded at 24 h (Table II). This complex response illustrated the importance to perform time-dependent studies with this compound.

The GSH redox cycle is an important system for the maintenance of the normal redox status in cells and for their protection from potential oxidants (21). FBZC caused a strong and rapid decrease in GR activity over time, especially at short incubation times, where a near 80% inhibition was observed. Total glutathione levels also decreased in parallel, then were no different from the control at 12 h, and at 24 h they were strongly impaired to about 30% that of control levels. GST, which is an enzyme that uses glutathione as a substrate to detoxify xenobiotics, was significantly activated, by approximately 30%, at short incubation times, but afterwards its action was inhibited until cells eventually died (Table II).

In neoplastic cells one of the main routes for the utilization of carbohydrates is the pentose phosphate pathway (PPP) (22), with its particularly important oxidative branch, which generates NADPH to be used as a reducing agent in the antioxidant cycles that counteract the deleterious effects of free radicals. FBZC deregulated the enzymes of the oxidative branch of the PPP (Table II).

Discussion

In spite of the availability of a diverse group of antineoplastic agents for use in the treatment of breast cancer, unwanted effects have been reported, while their efficacy is also often less than optimal (2). Thus, the discovery of new compounds is of high priority. Benzothiazoles are heterocyclic analogs with *in vitro* antineoplastic activity towards some types of human cancer

Table II. *Effect of N'-formyl-2-(5-nitrothiophen-2-yl)benzothiazole-6-carbohydrazide (FBZC) (5.4 μg/mL) on different antioxidant parameters over time, in breast cancer MCF7 cells. Results are expressed as the mean±SEM of four independent experiments.*

	3 h		6 h		12 h		24 h	
Parameter	CTR	FBZC	CTR	FBZC	CTR	FBZC	CTR	FBZC
SOD (U/mL*mg)	0.66±0.01	0.48±0.01***	0.66±0.01	0.48±0.02**	0.65±0.03	0.49±0.01**	0.66±0.02	0.51±0.03*
CAT (U/mg*100)	7.22±0.59	10.36±0.56**	7.19±0.78	12.7±0.01**	7.20±0.52	23.32±2.86***	7.20±0.72	3.71±0.22**
GR (U/mg)	4.91±0.10	1.17±0.12***	4.81±0.30	1.04±0.16***	4.76±0.28	2.72±0.07**	4.65±0.32	3.31±0.06*
GST (U/mg)	0.66±0.01	0.84±0.01***	0.67±0.01	0.79±0.01***	0.64±0.01	0.73±0.01***	0.64±0.01	0.58±0.02**
GSH+GSSG (mM/mg)	79.06±0.94	67.08±1.05**	78.01±1.11	70.68±0.94**	81.35±0.88	81.41±0.86	79.16±0.98	18.71±1.99***
G6PDH (U/mL*mg)	3.41±0.11	4.12±0.18*	3.40±0.15	3.70±0.22	3.49±0.18	3.68±0.13	3.56±0.15	3.30±0.09
6PGDH (U/mL*mg)	0.70±0.03	0.61±0.01*	0.74±0.02	0.78±0.04	0.69±0.03	0.81±0.02*	0.76±0.02	0.96±0.04*

SOD: Superoxide dismutase; CAT: catalase; GR: glutathione reductase; GST: glutathione-S-transferase; GSH: reduced glutathione; GSSG: oxidized glutathione; G6PDH: glucose-6-phosphate dehydrogenase; 6PGDH: 6-phosphogluconate dehydrogenase. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ compared to vehicle control (CTR).

cells (23). It has been described that the mechanisms of action of this chemical moiety could be related to oxidative stress and by activation of the apoptosis machinery (24). On the other hand, nitrothiophenes have also demonstrated different biological activities, such as potential antitumor and antibiotic actions, possibly involving nucleophilic attack by intracellular thiols and DNA damage (25).

Cellular macromolecules such as proteins, lipids and DNA are prone to alterations and damage by ROS and oxidative stress and these events could be exploited for the development of potential antitumor compounds as ROS inducers or as inhibitors of different antioxidant enzymes. Abietyl-isothiocyanate (ABITC) and (–)-epigallocatechin-3-gallate (EGCG) are potential antitumor agents that induce ROS generation and subsequent apoptosis (26, 27). In cancer cells, the oxidative stress induced by compounds is superimposed on the intrinsic stress, resulting in a more potent ROS-mediated cytotoxic process that preferentially inhibits tumor cell proliferation or kills these cells (28). In this context, and in the search for new anticancer compounds, a benzothiazole derivative that is conjugated with 5-nitrothiophene, namely FBZC, was synthesized and was evaluated as a potential antitumor against human breast cancer *in vitro*.

Our results demonstrated that FBZC induced cytotoxicity in a dose- and time-dependent manner in MCF7 cells, showing a more active response than the regular antineoplastic doxorubicin. This analogue also demonstrated a superior selectivity towards tumor than non-tumor cells, compared to the positive-control agents. It is important to note that the effects were partially reversed by the common antioxidants trolox and NAC, suggesting that the toxic effects of FBZC were mediated by ROS. To confirm this hypothesis, our results also showed that these reactive molecules and lipid peroxidation were significantly raised over time. Thus, FBZC induced an oxidative environment at its cytotoxic IC_{50}. This was also previously described for another benzothiazole, the 2-Acetyl-3-(6-methoxybenzothiazo)-2-yl-amino-acrylonitrile (AMBAN), which led to leukemia cell death due to elevation of ROS whose overproduction can stimulate chain reactions by interacting with different cellular macromolecules, contributing to cellular dysfunction and ultimately, cell death (8).

FBZC also inhibited the antioxidant enzymes SOD and GR in short term incubations. Both manganese SOD and GR are inhibited by nitration of critical tyrosine residues (29, 30). Thus, the nitro group ($–NO_2$) of FBZC might be responsible for such effects. It is possible that the strong inhibition of GR observed using FBZC could, in addition, be due to binding of FBZC through its 5-nitrothiopen moiety, to the interface dimer site of GR (31). The inhibition of GR by this compound was particularly striking due to its magnitude (around 80%) and rapid onset. Such inhibition impairs the GSH redox cycle, where glutathione disulfide is reconverted to GSH by GR. The oxidation of GSH by H_2O_2 or lipid hydroperoxides is catalyzed by glutathione peroxidases, while GSH can also be conjugated with electrophilic agents in a reaction catalyzed by endogenous GST. Indeed, the strong inhibition of GR may have impaired the detoxification of H_2O_2 and of lipid hydroperoxides by glutathione peroxidase, due to the lack of GSH. This effect could be worsened by the strong increase in GST activity observed at 3 h, since the available GSH would be conjugated with reactive metabolites, including lipid peroxidation products and FBZC, to detoxify them through GST. These results are in concordance with those for other azole compounds, since thiazole and benzothiazole induced activity of and are detoxified by GST (32).

The impairment of the GSH-dependent H_2O_2 elimination by inhibition of GR could be compensated by the strong (about 3-fold) and rapid up-regulation of catalase, induced by FBZC. Since in MCF-7 cells, glutathione peroxidases and catalase contribute together, equally, for the elimination of

H_2O_2 (33), it is probable that a 3-fold up-regulation of catalase not only compensates for the impairment of the GSH system, but it may result from an adaptive response triggered by an increased production of H_2O_2, caused by FBZC. The near three-fold increase in the DCFH measurement of ROS, observed after 2 h incubation with FBZC, further supports this notion. Thus, the inhibition of antioxidant enzymes combined with the increase in the production of ROS may justify the highly cytotoxic effect of FBZC on MCF-7 cells.

The up-regulation of catalase triggered by FBZC illustrates an adaptive response in which cells respond to oxidative stress by inducing antioxidant defences. In this regard, it is interesting to note that SOD was inhibited by FBZC in a steady fashion throughout the incubation. In this case, the adaptive response was unable to counteract this inhibition. This may be particularly important since inhibition of SOD causes accumulation of cellular superoxide anion and perhydroxyl, leading to oxidative damage of mitochondrial membranes, inducing the release of cytochrome c from mitochondria, thus causing apoptosis of tumor cells (34). In addition, SOD activity is related to a decrease of breast tumor cell growth and proliferation both *in vitro* and *in vivo* (35); thus, the inhibitory activity of FBZC on this enzyme, observed here, may be relevant for its potential antitumor action.

In conclusion, FBZC showed pro-oxidative effects on MCF7 cells by increasing ROS and TBARs, along with causing inhibition of SOD and GR. These responses led to a remarkable deregulation of the cell antioxidant profile which triggered oxidative stress, leading to subsequent cell death. The multiple target effects of this compound could explain its superior selective toxicity towards tumor cells, compared to regular antineoplastics *in vitro,* underlying its potential as a new antitumor agent.

Acknowledgements

This study was supported through grants PTDC/QUI/69466/2006 and PEst-OE/QUI/UI0612/2011 from Fundação para a Ciência e Tecnologia (FCT), Portugal and grant CDCH-UCV no. PG-06-7548-2009/1 from Central University of Venezuela. JRR acknowledges a postdoctoral fellowship (SFRH/BPD/64619/2009) from FCT.

References

1 DeSantis C, Siegel R, Bandi P and Jemal A: Breast cancer statistics, 2011. CA Cancer J Clin *61*: 409-418, 2011.

2 Stebbing J and Ellis P: An overview of drug development for metastatic breast cancer. Br J Nurs *21*: S18-S22, 2012.

3 Hu WP, Chen YK, Liao CC, Yu HS, Tsai YM, Huang SM, Tsai FY, Shen HC, Chang LS and Wang JJ: Synthesis, and biological evaluation of 2-(4-aminophenyl)benzothiazole derivatives as photosensitizing agents. Bioorg Med Chem *18*: 6197-6207, 2010.

4 Christodoulou MS, Colombo F, Passarella D, Ieronimo G, Zuco V, De Cesare M and Zunino F: Synthesis and biological evaluation of imidazolo[2,1-b]benzothiazole derivatives, as potential p53 inhibitors. Bioorg Med Chem *19*: 1649-1657, 2011.

5 Dubey R, Shrivastava PK, Basniwal PK, Bhattacharya S and Moorthy NS: 2-(4-Aminophenyl) benzothiazole: a potent and selective pharmacophore with novel mechanistic action towards various tumor cell lines. Mini Rev Med Chem *6*: 633-637, 2006.

6 Brunton VG, Kelland LR, Lear MJ, Montgomery GJ, Robertson JH, Robins DJ, Queen J and Workman P: Synthesis and biological evaluation of a series of tyrphostins containing nitrothiophene moieties as possible epidermal growth factor receptor tyrosine kinase inhibitors. Anticancer Drug Des *11*: 265-295, 1996.

7 Morley JO and Matthews TP: Studies on the biological activity of some nitrothiophenes. Org Biomol Chem *4*: 356-366, 2006.

8 Repicky A, Jantova S and Cipak L: Apoptosis of murine L1210 leukemia cells induced by 2-acetyl-3-(6-methoxybenzothiazo)-2-ylamino-acrylonitrile involves ROS-mitochondrial mediated death signalling and activation of p38 MAPK. Acta Chimica Slovaca *3*: 15-28, 2010.

9 Pelicano H, Carney D and Huang P: ROS stress in cancer cells and therapeutic implications. Drug Resist Updat *7*: 97-110, 2004.

10 Charris J, Camacho J, Ferrer R, Lobo G, Barazarte A, Gamboa N, Rodrigues J and López S: A convenient route to 2-substituted benzothiazole-6-carboxylic acids using nitrobenzene as oxidant J Chem Res *12*: 769-770, 2006.

11 Nociari MM, Shalev A, Benias P and Russo C: A novel one-step, highly sensitive fluorometric assay to evaluate cell-mediated cytotoxicity. J Immunol. Methods *213*: 157-167, 1998.

12 Li JJ, Tang Q, Li Y, Hu BR, Ming ZY, Fu Q, Qian JQ and Xiang JZ: Role of oxidative stress in the apoptosis of hepatocellular carcinoma induced by combination of arsenic trioxide and ascorbic acid. Acta Pharmacol Sin *27*: 1078-1084, 2006.

13 Ohkawa H, Ohishi N and Yagi K: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem *95*: 351-358, 1979.

14 McCord JM and Fridovich I: Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem *244*: 6049-6053, 1969.

15 Aebi H: Catalase *in vitro*. Methods Enzymol *103*: 121-126, 1984.

16 Tietze F: Enzymatic method for the quantitative determination of nanogram amounts of total and oxidized glutathione: Applications to mammalian blood and other tissues. Anal Biochem *27*: 502-522, 1969.

17 Goldberg DM and Spooner RJ: Glutathione reductase *In*: Methods of Enzymatic Analysis. Bergmeyer HU (ed.). Verlag Chemie Deerfield Beach, Florida, pp. 258-265, 1983.

18 Rice K, Penketh P, Shyam K and Sartorelli A: Differential inhibition of cellular glutathione reductase activity by isocyanates generated for the antitumor prodrug cloretazine and BCNU. Biochem Pharmacol *69*: 1463-1472, 2005.

19 Tian WN, Leigh DB, Pignatare JN and Stanton RC: Signal transduction proteins that associate with the platelet-derived growth factor (PDGF) receptor mediate the PDGF-induced release of the glucose-6-phosphate dehydrogenase from the permeabilized cells. J Biol Chem *269*: 14798-14805, 1994.

20 Bradford M: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem *72*: 248-254, 1976.

21 Meister A: Selective modification of glutathione metabolism. Science *220*: 472-477, 1983.
22 Kit S: The role of the hexosemonophosphate shunt in tumors and lymphatic cells. Cancer Res *16*: 70-76, 1956.
23 Wells G, Bradshaw TD, Diana P, Seaton A, Shi DF, Westwell AD and Stevens MFG: Antitumour benzothiazole. Part 10: The synthesis and antitumour activity of benzothiazole substituted quinol derivatives. Bioorg Med Chem Lett *10*: 513-515, 2000.
24 Kok SH, Gambari R, Chui CH, Yuen MC, Lin E, Wong RS, Lau FY, Cheng GY, Lam WS, Chan SH, Lam KH, Cheng CH, Lai PB, Yu MW, Cheung F, Tang JC and Chan AS: Synthesis and anticancer activity of benzothiazole containing phthalimide on human carcinoma cell lines. Bioorg Med Chem *16*: 3626-3631, 2008.
25 Morley JO and Matthews TP: Studies on the biological activity of some nitrothiophenes. Org Biomol Chem *4*: 359-366, 2006.
26 Horan TC, Zompa MA, Seto CT, Kim K, Moore RG and Lange TS: Description of the cytototoxic effect of a novel drug abietyl-isothiocyanate on endometrial cancer cell lines. Invest New Drugs In Press Aug 2, 2011.
27 Li GX, Chen YK, Hou Z, Xiao H, Jin H, Lu G, Lee MJ, Liu BA, Guan F, Yang Z, Yu A and Yang CS: Pro-oxidative activities and dose–response relationship of (−)-epigallocatechin-3-gallate in the inhibition of lung cancer cell growth: a comparative study *in vivo* and *in vitro*. Carcinogenesis *31*: 902-910, 2010.
28 Manda G, Nechifor MT and Neagu TM: Reactive oxygen species, cancer and anticancer therapies. Curr Chem Biol *3*: 342-366, 2009.
29 Savvides SN, Scheiwein M, Bohme CC, Arteel GE, Karplus PA, Becker K and Schirmer RH: Crystal structure of the antioxidant enzyme glutathione reductase inactivated by peroxynitrite. J Biol Chem *277*: 2779-2784, 2002.
30 Yamakura F, Taka H, Fujimura T and Murayama K: Inactivation of human manganese-superoxide dismutase by peroxynitrite is caused by exclusive nitration of tyrosine 34 to 3-nitrotyrosine. J Biol Chem *237*: 14085-14089, 1998.
31 Vega-Teijido M, Caracelli I and Zukerman-Schpector J: Conformational analyses and docking studies of a series of 5-nitrofuran- and 5-nitrothiophen-semicarbazone derivatives in three possible binding sites of trypanothione and glutathione reductases. J Mol Graph Model *24*: 349-355, 2006.
32 Kim SG and Cho MK: Expression of glutathione-*S*-transferases Ya, Yb1, Yb2, Yc1 and Yc2 and microsomal epoxide hydrolase gene by thiazole, benzothiazole and benzothiadiazole. Biochem Pharmacol *52*: 1831-1841, 1996.
33 de Oliveira-Marques V, Cyrne L, Marinho HS and Antunes F: A quantitative study of NF-kappaB activation by H_2O_2: relevance in inflammation and synergy with TNF-alpha. J Immunol *178*: 3893-3902, 2007.
34 Huang P, Feng L, Oldham EA, Keating MJ and Plunkett W: Superoxide dismutase as a target for the selective killing of cancer cells. Nature *407*: 390-395, 2000.
35 Weydert CJ, Waugh TA, Ritchie JM, Iyer KS, Smith JL, Li L, Spitz DR and Oberley LW: Overexpression of manganese or copper-zinc superoxide dismutase inhibits breast cancer growth. Free Radic Biol Med *41*: 226-237, 2006.

Received April 22, 2012
Revised May 23, 2012
Accepted May 24, 2012