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Article

Synthesis and cytotoxicity evaluation of thiosemicarbazones and their thiazole derivatives

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The aims of this study were to synthesize a series of thiosemicarbazones and their thiazole derivatives, to investigate their cytotoxic activity against three human cancers and normal (Vero cells) cell lines, and to evaluate the pro-apoptotic potential of the most active compounds. Materials and Methods: The thiosemicarbazones were obtained by reacting an aromatic aldehyde with thiosemicarbazide (yield 71-96%), which were subjected to a cyclization with α-bromoacetophenone to yield the required thiazole heterocycles (yield 63-100%). All the synthesized compounds were screened at 50 μM concentration against three cell lines representing HL60 (promyelocytic leukemia), Jurkat (acute lymphoblastic leukemia), and MCF-7 (breast cancer). The pro-apoptotic effect was measured by flow cytometry as the percentage of cells with hypodiploid DNA. Results: Three thiazole compounds showed activity against at least one tumor cell line (IC_{50} = 43-76 μM) and low cytotoxicity against Vero cells (IC_{50} > 100 μM). The most active compound of this series induced 91% and 51% DNA fragmentation in HL60 and MCF-7 cell lines, respectively, suggesting that this compound triggered apoptosis in these cells. Conclusion: Among the synthesized compounds, one in particular was found to exert antiproliferative and pro-apoptotic activity on tumor cells and can be considered promising as a lead molecule for the design of new analogues with improved activity.

Uniterms: Thiosemicarbazones/synthesis. Thiosemicarbazones/cytotoxic activity. Thiazoles/derivatives/synthesis. Thiazoles/derivatives/cytotoxic activity.

O estudo teve como objetivo a síntese de uma série de tiossemicarbazonas e seus derivados tiazólicos e a avaliação da atividade citotóxica contra três linhagens de células tumorais humanas e células normais (Vero), a fim de se avaliar o potencial pró-apoptótico dos compostos mais ativos. As tiossemicarbazonas foram obtidas por reação entre um aldeído aromático e tiossemicarbazida (rend. 71-96%), as quais foram submetidas à ciclização com α-bromoacetofenona, fornecendo os heterociclos tiazólicos desejados (rend. 63-100%). Todos os compostos sintetizados foram testados na concentração de 50 μM contra três linhagens de células tumorais: HL60 (leucemia promielocítica), Jurkat (leucemia linfoblástica aguda) e MCF-7 (câncer de mama). O efeito pró-apoptótico foi avaliado por citometria de fluxo como porcentagem de células com DNA hipodiplóide. Três compostos tiazólicos foram ativos contra, pelo menos, uma linhagem tumoral (CI_{50}=43-76 μM), com baixa citotoxicidade contra células Vero (CI_{50} > 100 μM). O composto mais ativo dessa série induziu fragmentação do DNA de 91% e 51% nas linhagens HL60 e MCF-7, respectivamente, sugerindo que este composto ativou a apoptose nessas células. Dentre os compostos sintetizados, um em particular apresentou atividade antiproliferativa e pró-apoptótica em células tumorais e pode ser considerado composto protótipo promissor na busca por novos análogos com atividade melhorada.

Unitermos: Tiossemicarbazonas/síntese. Tiossemicarbazonas/atividade citotóxica. Tiazol/derivados/síntese. Tiazol/derivados/atividade citotóxica.

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INTRODUCTION

Thiosemicarbazones and their thiazole heterocyclic derivatives are of great importance in pharmacology due to their broad range of biological activities, such as anticonvulsant, antimicrobial, and antiparasitic activities (Gaikwad, Patil, Bobade, 2013; Souza *et al.*, 2013; Bharti *et al.*, 2010; Siddiqui *et al.*, 2009; Oliveira *et al.*, 2008a; Souza, 2005; Dimmock *et al.*, 1991).

The synthesis of thiazole compounds from thiosemicarbazones is of particular interest in medicinal chemistry due to their ease of synthesis, low cost, good yields, and the possibility of obtaining a wide diversity of derivatives, allowing the modulation of pharmacokinetics and optimization of biological activity. In addition, this class of heterocyclic compounds has demonstrated great potential as anticancer agents with a broad spectrum of action against different types of cancer cell lines (Abdel-Maksoud *et al*., 2016; Turan-Zitouni *et al*., 2016; Morigi *et al.*, 2015; Zhao *et al.*, 2015).

In this context, and following the work developed by our research group in the development of new compounds with antitumor activity (Lages *et al.*, 2013; Soares *et al.*, 2010), the present study aimed to synthesize thiosemicarbazones and their thiazole derivatives (Figure 1) and to evaluate their antiproliferative activity against three human tumor cell lines: HL60 (promyelocytic leukemia), Jurkat (acute lymphoblastic leukemia), and MCF-7 (breast cancer). The cytotoxic activity against Vero cell lines (normal monkey kidney cells) was also aimed to investigate the selectivity of the compounds to nontumor cells, which have been used as a preliminary model to assess the selectivity of new bioactive compounds (Lavrado *et al.*, 2010; Küster *et al.*, 2012).

Apoptosis or programmed cell death is an important parameter in drug-induced toxicity (Weber *et al.*, 2004). Deregulation of apoptosis-regulating genes can lead to diseases such as cancer and has been related to pathogenesis and cancer progression (Fischer, Schulze-Osthoff, 2005). Drugs that restore the normal apoptotic pathways have the potential for effectively treating cancers that depend on aberrations of the apoptotic pathway to stay alive (Fesik, 2005). Therefore, the search for compounds capable of inducing apoptosis in tumor cells is of great interest in the development of novel drugs for cancer treatment. In this context, we also evaluated the apoptotic potential of the active compounds measuring the increase of diploid DNA content of susceptible cells by flow cytometry, as a preliminary study to gain insights into their action mechanism comparing with etoposide, a pro-apoptotic drug used in the clinic.

MATERIAL AND METHODS

General procedures

Melting points were determined on a Microquímica MQAPF 301 apparatus and are uncorrected. FT-IR spectra were recorded using a Perkin Elmer Spectrum One infrared spectrometer and absorptions are reported as wave numbers (cm^{-1}). All NMR spectra were recorded on a Bruker Avance DPX 200 spectrometer (200 MHz). Chemical shifts are given in δ (ppm) scale and *J* values are given in Hz. All reagents of analytical grade were obtained from commercial suppliers and used without previous purification. Reactions were monitored by TLC using silica gel coated plates and different solvents solutions as the mobile phase. The synthesis of the compounds **1a** and **1c** (Rajak *et al.*, 2011), **1b** (Tenório, Carvalho, Pessanha, 2005), **1d** (Grammaticakis, Sorbonne, 1959), **1e** (Behnisch, Mietzsch, Schmidt, 1955), **1f** (Oliveira *et al.*, 2008b), **2a** and **2c** (Shih, Su, Wu, 2007), **2b** (Maccioni *et al.*, 2003) and **2e** (Bilinski, Tyburczyk, Urban, 1961) was previously reported in the literature.

General procedure: synthesis of thiazole heterocycles (Dimmock et al., 1991)

To a solution of 1 equiv. of thiosemicarbazones (**1a-1f**) in isopropyl alcohol was added 1 equiv. of 2-bromoacetophenone or 2-bromo-4'-methoxyacetophenone, and the resulting mixture was kept under reflux and magnetic stirring (approximately 90 minutes or indicated time). After cooling to room temperature, part of the solvent was removed under

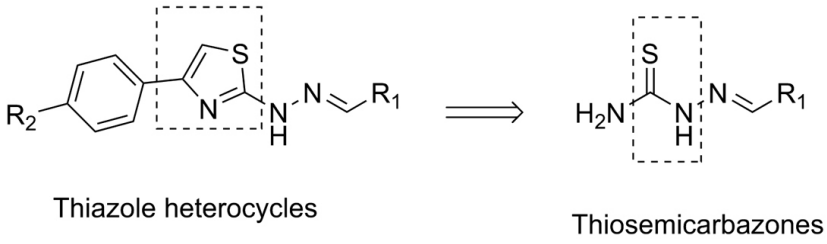


FIGURE 1 - General structure of synthesized thiosemicarbazones and hetetocycles.

reduced pressure and the formed precipitate was filtered, washed with saturated solution of $NaHCO_3$ followed by cold distilled water.

*4-Phenyl-2-(2-(quinolin-4-ylmethylene)hydrazinyl) thiazole (**2d**).*

The reaction was carried out following the general procedure described above affording 0.141 g of a brownish solid (65% yield). Mp 112.0-114.5 ºC. IR, ($\bar{\upsilon}$/cm^{-1}): 3452 (NH), 1659 (C=N), 1619, 1594, 1551, 1452 (C=C aromatic). ^{1}H NMR (200 MHz, DMSO-d_6), δ/ppm: 12.68 (1 H, s, NH); 8.94 (1 H, d, quinoline); 8.93-8.70 (2 H, m, quinoline and CH=N); 8.07 (1 H, d, H-8 quinoline); 7.90-7.68 (5 H, m, quinoline and ArH); 7.46-7.27 (4 H, m, ArH and H-thiazole). ^{13}C NMR (50 MHz, DMSO-d_6), δ/ppm: 167.7 (SC=N); 150.7; 150.2; 148.3 (C-4 thiazole, C-2, C-9 quinoline); 138.0 (CH=N); 137.4 (C-4 quinoline); 134.5 (C-Ph); 129.7, 129.6, 128.6 (C-Ph), 127.7, 127.4, 125.6 (C-Ph), 124.2 (C-5, C-6, C-7, C-8 quinoline and C-Ph); 124.3 (C-10 quinoline); 119.0 (C-3 quinoline); 104.5 (CH-thiazole).

*2-(2-((5-(4-Nitrophenyl)furan-2-yl)methylene) hydrazinyl)-4-phenylthiazole (**2f**).*

The reaction was carried out following the general procedure described above affording 0.066 g of an orange solid (92% yield). Mp 208.3 ºC with decomposition. IR, ($\bar{\upsilon}$/cm^{-1}): 1597 (C=N), 1580, 1562, 1483 (C=C aromatic), 1508, 1343 (NO_2). ^{1}H NMR (200 MHz, DMSO-d_6), δ/ppm: 12.33 (1 H, s, NH); 8.29 (2 H, broad s., $ArNO_2$); 7.98 (3 H, broad s., CH=N + $ArNO_2$); 7.85 (2 H, br. s., ArH); 7.42-7.37 (5 H, m, 2 x ArH, H-thiazole and H-5 furan); 7.00 (1 H, s, H-2 furan). ^{13}C NMR (50 MHz, DMSO-d_6), δ/ppm: 167.6 (SC=N); 151.6, 151.1, 150.4 (C-4 thiazole, C-2 and C-5 furan); 146.1 (C-$ArNO_2$); 135.3, 134.5 (C-$ArNO_2$ and C-Ph); 130.7 (CHC=N); 128.6 (C-Ph), 127.6 (C-Ph); 125.9 (C-Ph); 124.5, 124.2 (C-$ArNO_2$); 114.2 (C-4 furan); 112.6 (C-3 furan); 103.9 (CH- thiazole).

*4-(4-Methoxyphenyl)-2-(2-(3-nitrobenzylidene) hydrazinyl)thiazole (**2g**).*

The reaction was carried out following the general procedure described above affording 0.168 g of a yellow solid (quantitative yield). Mp 190.0-193.3 ºC. IR, ($\bar{\upsilon}$/cm^{-1}): 3314 (NH), 1587 (C=N), 1527, 1346 (NO_2), 1511, 1463 (C=C aromatic). ^{1}H NMR (200 MHz, DMSO-d_6), δ/ppm: 8.45 (1 H, s, $ArNO_2$); 8.20-8.16 (2 H, m, $ArNO_2$ and HC=N); 8.07 (1 H, d, $ArNO_2$); 7.79-7.67 (3 H, m, $ArNO_2$ and $ArOCH_3$); 7.19 (1 H, s, H-thiazole); 6.97 (2 H, d, $ArOCH_3$); 3.77 (3 H, s, OCH_3). ^{13}C NMR (50 MHz, DMSO-d_6), δ/ppm: 167.9 (SC=N); 159.0 (C-$ArOCH_3$); 148.3 (C-4 thiazole); 151.5 (C-$ArNO_2$); 139.4 (CH=N); 136.2 (C-$ArNO_2$); 132.4 (C-$ArNO_2$); 130.4 (C-$ArNO_2$); 127.0 (C-$ArOCH_3$); 126.9 (C-$ArOCH_3$); 123.4 (C-$ArNO_2$); 120.3 (C-$ArNO_2$); 114.0 (C-$ArOCH_3$); 102.2 (CH-thiazole); 55.2 (OCH_3).

*4-(4-Methoxyphenyl)-2-(2-(quinolin-4-ylmethylene) hydrazinyl)thiazole (**2h**).*

The reaction was carried out following the general procedure described above affording 0.146 g of a brownish solid (94% yield). Mp 97.7 ºC with decomposition. IR, ($\bar{\upsilon}$/cm^{-1}): 3420 (NH), 1580 (C=N), 1552, 1505, 1488 (C=C aromatic). ^{1}H NMR (200 MHz, DMSO-d_6), δ/ppm: 12.67 (1 H, s, NH); 8.94 (1 H, d, H-quinoline); 8.69 e 8.64 (2 H, m, H-quinoline and CH=N); 8.08 (1 H, d, H-quinoline); 7.82-7.68 (5 H, m, H-quinoline and $ArOCH_3$); 7,24 (1 H, s, H-thiazole); 6.97 (2 H, d, $ArOCH_3$); 3.78 (3 H, s, OCH_3). ^{13}C NMR (50 MHz, DMSO-d_6), δ/ppm: 168.0 (SC=N); 159.3 (C-$ArOCH_3$); 150.9, 150.4, 148.5 (C-4 thiazole, 2 x C-quinoline); 138.2 (C-quinoline); 137.9 (CH=N); 130.1, 130.0 (2 x C-quinoline); 127.8 (C-quinoline); 127.7 (C-$ArOCH_3$); 127.3 (C-quinoline); 124.7 (C-$ArOCH_3$); 126.6 (C-quinoline); 119.3 (C-quinoline); 114.4 (C-$ArOCH_3$); 102.7 (CH-thiazole); 55.5 (OCH_3).

*4-(4-Methoxyphenyl)-2-(2-((5-(4-nitrophenyl)furan-2-yl)methylene)hydrazinyl)thiazole (**2i**).*

The reaction was carried out following the general procedure described above affording 0.080 g of a dark red solid (quantitative yield). Mp 202.9 ºC with decomposition. IR, ($\bar{\upsilon}$/cm^{-1}): 3372 (NH), 1618 (C=N), 1608, 1608, 1487 (C=C aromatic), 1509, 1331 (NO_2). ^{1}H NMR (200 MHz, DMSO-d_6), δ/ppm: 8.26 (2 H, broad s., $ArNO_2$); 7.99-7.96 (3 H, m, CH=N + $ArNO_2$); 7.75 (2 H, broad s., $ArOCH_3$); 7.42 (1 H, s, H-thiazole); 7.19 (1 H, s, H-4 furan); 6.99 (4 H, broad s., NH, H-3 furan, $ArOCH_3$); 3.78 (3 H, s, OCH_3). ^{13}C NMR (50 MHz, DMSO-d_6), δ/ppm: 167.7 (SC=N); 159.0 (C-$ArOCH_3$); 151.8, 151.0, 149.2 (C-4 thiazole, C-2 and C-5 furan); 146.1 (C-$ArNO_2$); 135.2 (C-$ArNO_2$); 131.5 (CHC=N); 126.9 (C-$ArOCH_3$); 126.7 (C-$ArOCH_3$); 124.5, 124.3 (C-$ArNO_2$); 114.7 (C-4 furan); 114.0 (C-$ArOCH_3$); 112.8 (C-3 furan); 102.1 (CH thiazole); 55.2 (OCH_3).

Biological assays

Cell lines

Jurkat (human T cells leukemia), HL-60 (human promyelocytic leukemia) and MCF-7 (breast carcinoma) cell lines were generously donated by Dr. Gustavo P.

Amarante-Mendes of Universidade de São Paulo-USP and Professor Alfredo Goes (Universidade Federal de Minas Gerais). Vero cells (derived from the kidney of an African green monkey) were donated by Erna Kroon (Universidade Federal de Minas Gerais).

Leukemic cells were kept in a RPMI 1640 medium (Sigma Aldrich, USA) supplemented with 1% antibiotic solution (100 U/mL penicillin and 100 µg/mL streptomycin - GIBCO BRL, Grand Island, NY), buffered with 2 mM of L-glutamina (GIBCO UK, Grand Island, NY) 10% of fetal bovine serum (GIBCO BRL, Grand Island, NY), being incubated under a 5% CO_2 atmosphere at 37 °C. MCF-7 cells were kept in D-MEM (Sigma Aldrich, USA), supplemented as described for leukemic cells but 5% of fetal bovine serum. All cultures were maintained at 37 ºC in a humidified incubator with 5% CO_2 and 95% air. The media were changed twice weekly and they were regularly examined.

Cytotoxicity assays

The screening assays to evaluate the cytotoxic effects of the thiosemicarbazones and their thiazole derivatives were performed in 96 well plate using HL60 and Jurkat cells at 50,000 and 100,000 cells/well, respectively. MCF-7 and Vero cells were seeded at 10,000 cells/well. The plates were pre-incubated for 24 h at 37 ºC to allow adaptation of cells prior to the addition of the compounds at 50 µM. Treated and untreated cells (DMSO 0.5%) were incubated for 48 hours and compounds that inhibited the cell viability greater than 50% had the IC_{50} values obtained graphically from concentration-effect curves using Prism 5.0 (GraphPad Software Inc.). Etoposide was used as positive control under the same experimental conditions. All substances were evaluated in two independent experiments, in triplicate. Cell viability was estimated by MTT assay.

In vitro cell viability assay - MTT assay

Viability assay was measured by MTT method (Mosmann, 1983). In brief, after 4 hours of the end of incubation of cells with different compounds, 20 µL of MTT solution (5mg/mL in phosphate-buffered saline) were added to each well, the supernatant was removed and 200 µL of 0.04 M HCl in isopropyl alcohol were added to solve the formazan crystal. Absorbance of the samples (treated and untreated) was measured at in a spectrophotometer at 570 nm. Data were expressed as perceptual of cell viability comparing with DMSO (0.5%). Values of absorbance of medium plus drugs, without cells were used to escape from false positive or false negative (Ulukaya, Colakogullari, Wood, 2004).

Determination of hypodiploid DNA content by using a hypotonic fluorochrome solution (HFS)

The proapoptotic potential of the most actives compounds was evaluated by quantifying of hypodiploid DNA content, as described by Nicoletti and coworkers (1991). The cells were treated with compounds (50 µM) and incubated for 24 hours. A total of 2.0 X 10^5 cells were resuspended in 0.3 ml of hypotonic fluorochrome solution (50 µg/mL propidium iodide and 0.1% Triton X-100 in 0.1% sodium citrate). After 2–4 h at 4 °C in the dark, the fluorescence of individual nuclei was measured using a FACS flow cytometer (Becton–Dickinson, Mountain View, CA).

RESULTS AND DISCUSSION

The thiosemicarbazones (**1a-f**) and their cyclic derivatives (**2a-i**) were synthesized as shown in Figure 2. Initially, the thiosemicarbazones were obtained using a classic methodology, by reacting an aromatic aldehyde with thiosemicarbazide. Then, the thiosemicarbazones were subjected to a cyclization reaction in the presence of α-bromoacetophenone. Non-substituted α-bromoacetophenone (R_2 = H) or *para* substituted by a methoxyl group (R_2 = OCH_3) were used for comparison purpose. The presence of a methoxyl group, besides improving compound solubility, could contribute as a possible hydrogen bond acceptor with the molecular target.

All compounds were tested at 50 µM against three human cancer cell lines: HL60 (promyelocytic leukemia), Jurkat (acute lymphoblastic leukemia), and MCF-7 (breast cancer) (Table I). The drug etoposide was utilized at 10 µM as positive control.

Three compounds were able to inhibit more than 50% of the growth of at least one of the cancer cell lines and then their IC_{50} values were determined. In addition, cytotoxicity of the active compounds was evaluated against African green monkey kidney epithelial cells (Vero cells), a model used to investigate possible toxicity against non-tumor cell lines (Küster *et al.*, 2012) (Table II).

Results from the initial screening allowed us to select three compounds (**2d**, **2f,** and **2h**) able to inhibit more than 50% of viability of breast carcinoma cells (MCF-7) (IC_{50} values 54, 43, and 76 µM, respectively). Among the tested compounds, only the thiazole **2h** exhibited activity against promyelocytic leukemia cells (HL-60) with an IC_{50} value of 43 µM. None of the compounds tested showed significant cytotoxic activity against Jurkat cells. The concentration of the three compounds that inhibited Vero

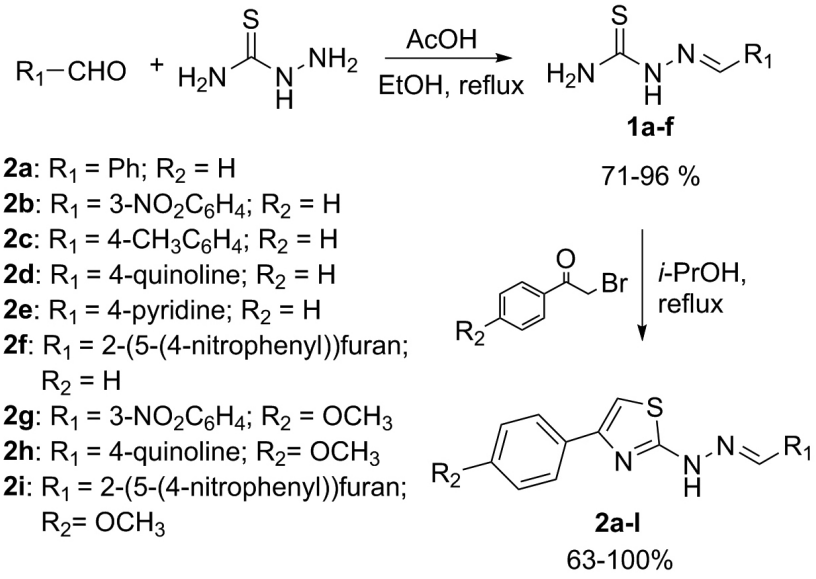


FIGURE 2 - Route of synthesis of the thiosemizarbazones and their corresponding thiazole heterocycles.

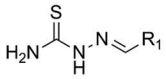
cell proliferation by 50% (IC_{50}) was greater than 100 μM, indicating low toxicity.

Accordingly with the results obtained, we were able to conclude that the presence of a thiazole ring is essential for activity against MCF-7 cells, since corresponding thiosemicarbazones **1d** and **1f**, at the same concentration, were less active. Furthermore, in some cases, the presence of a second heterocyclic ring, quinoline (**2d** and **2h**) or furan (**2f**), favored the cytotoxic activity. However, for the pyridine derivative **2e** and furan derivative **2i**, the presence of an additional heterocyclic ring did not result in a significant improvement in the activity. With respect to antiproliferative activity against HL-60 cells, comparison between **2d** and **2h** indicates the importance of the methoxyl group.

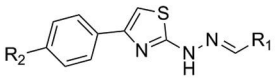
The effect of anticancer drugs on tumor cell lines such as HL60 and MCF-7 has been associated with apoptosis induction (Robinson, Shewach, 2001). In order to identify the pro-apoptotic potential of the more promising compounds (**2d**, **2f** and **2h)**, the hypodiploid DNA content, which permits the evaluation of DNA fragmentation of cells undergoing apoptosis, was determined. In this assay, propidium iodide intercalates into the base pairs of double-stranded DNA, allowing the quantification of subdiploid DNA content and of the cells in different phases of the cell cycle (vide experimental section for details). The results summarized in Table II show that compound **2h** induced a significant increase (90%) of subdiploid DNA content in HL-60 cells, similar to etoposide. Regarding the MCF-7 cells, the three compounds tested induced an increase of approximately 50% in the hypodiploid DNA. These data suggest that the cytotoxic effect of these compounds is related to the activation of the apoptosis pathways. It is noteworthy that etoposide exhibits low activity (IC_{50} > 100 μM) against MCF-7 cells.

The susceptibility of HL60 and MCF-7 to the thiazole compounds **2d**, **2f,** and **2h** can be correlated with p53 status, a tumor suppressor that is responsible for triggering distinct responses to cellular stress and for mediating several of the antiproliferative processes, including apoptosis induction in response to DNA damage, among others (Fridman, Lowe, 2003). Cancer cells that express only mutated p53 protein have shown differential sensitivity to therapeutic modalities such as chemotherapeutic drugs or ionizing radiation, mediated by its inability to recognize DNA damage and promote damage repair or apoptosis (Robinson, Shewach, 2001; Ju *et al.*, 1998). In the current investigation, the two lineages more susceptible to the new thiazole compounds possess different status of the p53 protein. HL60 (promyelocytic leukemia) lacks p53 (p53-null), which is very sensitive to various apopotic stimuli (Shimizu, Pommier, 1996), and MCF-7 (human breast carcinoma), a wild-type p53 (Robinson, Shewach, 2001). The two strains showed differential behavior towards the treatments with the three promising compounds. Whereas HL60 was sensitive only to **2h**, MCF-7 cells demonstrated more sensibility to the three compounds. Compound **2h** induced DNA fragmentation in both lineages (p53-null and wild-type). Since the HL-60 cell line does not express p53, it may be concluded that apoptosis induced by **2h** is not dependent on this pathway. Therefore, this thiosemicarbazone can trigger different mechanisms of cell death independent of p53, which needs to be further investigated.

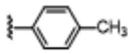
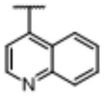
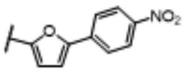
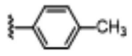
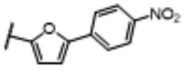
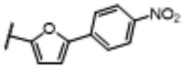
TABLE I - Cytotoxicity activity assay results of synthesized compounds against tumor cells



1a-f



2a-l

Compounds	R_1	R_2	% inhibition of cell proliferation		
			HL60	Jurkat	MCF-7
1a	Ph	-	5.9 ± 3.7	0	8.5 ± 3.5
1b		-	23.3 ± 6.0	16.0 ± 4.3	2.6 ± 3.5
1c		-	11.6 ± 5.0	6.2 ± 2.1	24.7 ± 0.4
1d		-	0	38.2 ± 10.5	28.9 ± 6.1
1e		-	0	0	10.9 ± 4.5
1f		-	7.5 ± 1.0	37.6 ± 6.5	11.0 ± 7.0
2a	Ph	H	9.2 ± 0.3	2.9 ± 2.3	19.3 ± 5.4
2b		H	0	15.4 ± 7.9	33.5 ± 3.6
2c		H	16.9 ± 4.2	0	4.3 ± 0.6
2d		H	45.7 ± 1.6	32.6 ± 15.6	**56.1 ± 0.1**
2e		H	26.0 ± 2.1	6.0 ± 4.9	44.9 ± 4.7
2f		H	26.6 ± 1.2	42.3 ± 13.2	**50.6 ± 4.2**
2g		OCH_3	33.6 ± 11.9	43.3 ± 1.1	39.6 ± 8.1
2h		OCH_3	**82.2 ± 4.8**	34.5 ± 12.1	**58.7 ± 3.3**
2i		OCH_3	37.7 ± 2.4	18.1 ± 16.9	13.8 ± 1.0
Etoposide	-	-	68.7 ± 11	54 ± 10	inactive

Compounds **2d** and **2f** induced an increase of DNA subdiploid in MCF-7 cells, but not in HL60 (p53-null). It has been described that exposure of wild-type p53 cells to DNA-damaging agents increases p53 protein levels leading to G1 or G2 cell cycle blockage or apoptosis induction (Robinson, Shewach, 2001). The cross-talking between p53 function and cytotoxicity is a complex process and dependent upon the particular cytotoxic agent, cell line, and

TABLE II - IC_{50} values and evaluation of pro-apoptotic potential of the compounds **2d**, **2f** e **2h**

Compounds	CI_{50} (μM)				% hypodiploid DNA	
	HL60	Jurkat	MCF-7	Vero	HL60	MCF-7
2d	>100	>100	54 ± 11	>100	ND*	**53.8 ± 4.2**
2f	>100	>100	43 ± 13	>100	ND*	**45 ± 16**
2h	43 ± 3	>100	76 ± 10	>100	**90.9 ± 2**	**50.9 ± 12**
Etoposide	16 ± 4	39 ± 9	>100	> 100	**86 ± 9**	**7.6 ± 3**

*ND = not determined

cell death pathways used. Our results also corroborated the data by (Tudor *et al.*, 2000), that demonstrated differences in susceptibility to drugs in HL60 and MCF-7 which has been attributed to qualitative changes in apoptotic protein expression, after exposure to agents with different mechanisms of action. Under our conditions, the positive control etoposide, used in the clinic, induced DNA fragmentation in HL60, but not in MCF-7. Shimizu and Pommier (1996) reported that topoisomerase inhibitors such as etoposide induce massive apoptosis in HL60 cells with the typical internucleosomal DNA fragmentation. Resistance to drugs, specifically to etoposide as observed in MCF-7, may arise as a result of alterations in target expression and activity, increased drug efflux, and alterations in DNA damage response mechanisms (Aktan *et al.*, 2014). Therefore, further experiments should be conducted to elucidate the mechanism/s involved in the cytotoxic effect of the new thiosemicarbazones on these cell lines.

CONCLUSION

A series of thiosemicarbazones and their thiazole derivatives were synthesized in this work. All compounds were evaluated *in vitro* against three human cancer cell lines. Among the tested compounds, the thiazole heterocycles **2d**, **2f** and **2h** exhibited moderate cytotoxicity activity against HL60 and/or MCF-7 cell lines (IC_{50} range: 43-76 M) and low cytotoxicity against Vero cells (normal cells) (IC_{50} > 100 μM). The potential pro-apoptotic of the three compounds was investigated, demonstrating a difference in induction of DNA fragmentation in HL60 and MCF-7 cells. Among the active compounds, **2h** displayed the best results in this preliminary study and can be considered a promising lead compound in the design of more potent analogues.

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