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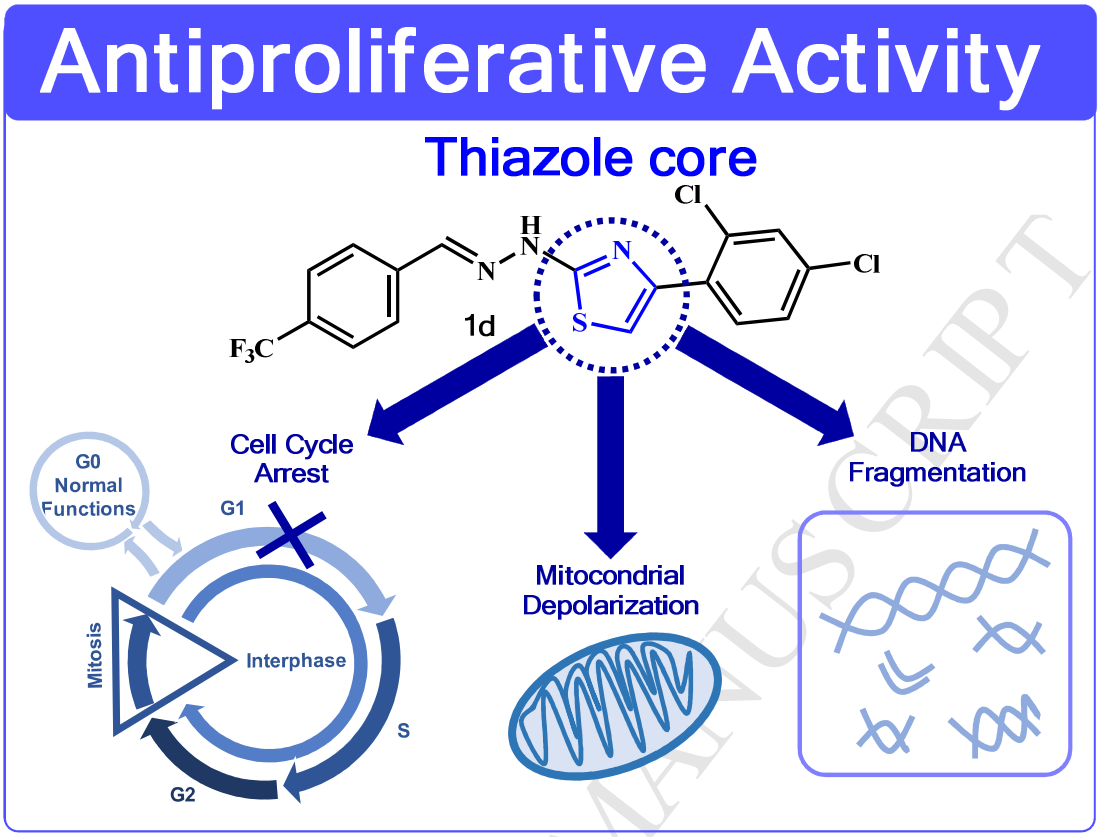
GRAPHICAL ABSTRACT



Synthesis, Anticancer Activity and Mechanism of Action of New Thiazole Derivatives

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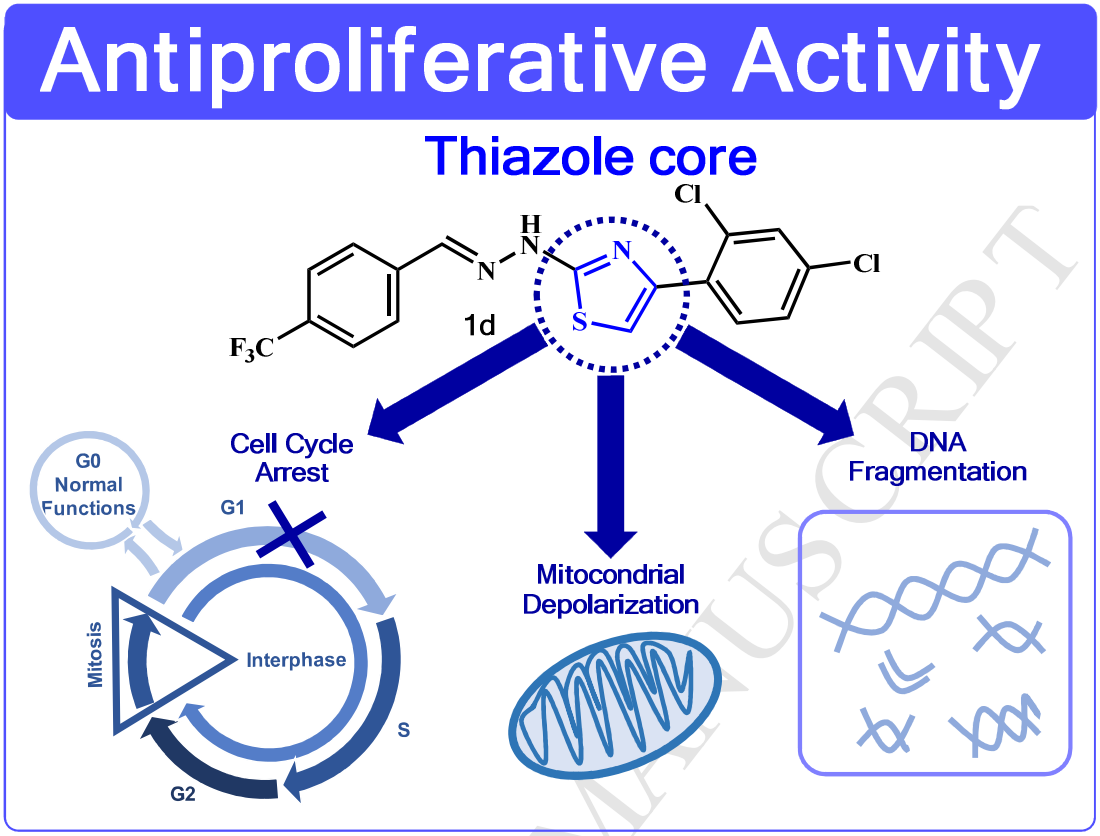
Abstract

Thiazole derivatives are recognized to possess various biological activities as antiparasitic, antifungal, antimicrobial and antiproliferative. The present work reports the synthesis of 22 new substances belonging to two classes of compounds: thiosemicarbazones and thiazoles, with the purpose of developing new drugs that present high specificity for tumor cells and low toxicity to the organism. A cytotoxic screening was performed to evaluate the performance of the new derivatives in five tumor cell lines. Eight compounds were shown to be promising in at least three tumor cell lines. These compounds had their IC_{50} determined within 72 hours and the activity structure ratio was assessed. The effect of the best compounds on PBMC and hemolytic activity assay was then evaluated. The compound **1d** was considered the most promising among the samples tested and its influence on cell cycle, DNA fragmentation and mitochondrial depolarization was evaluated.

Keywords

Cancer; Thiazoles; Cell cycle; DNA fragmentation; Mitochondrial depolarization

GRAPHICAL ABSTRACT



HIGHLIGHTS

The cytotoxic activity of 3 thiosemicarbazones and 19 new thiazoles was evaluated;

Eight compounds were shown to be promising in at least three tumor cell lines;

Compound **1d** induced mitochondrial depolarization;

Compound **1d** induced DNA fragmentation and arrest cell cycle in G1 phase;

Compound **1d** presented as a promising antitumor candidate.

1 Introduction

Cancer is one of the major public health problems in many countries around the world [1]. A complex disease of genetic trait has long been considered a process of autonomous cell death, resulting from the acquisition of successive mutations in oncogenes and tumor suppressor genes that result in a progressive proliferation and resistance to cell death [2, 3].

The standard treatment for cancer, although not specific, is based on surgery, radiotherapy, and chemotherapy. Often, surgical resection and radiotherapy are successful techniques in eradicating the primary tumor, although a problem commonly found is relapse of the disease due to residual tumor cells and/or metastasis. Therefore, these therapeutic strategies are usually followed by chemotherapy [4].

Although many antitumor agents are present in the literature and commercially available, such compounds exhibit high cytotoxicity and develop resistance, which may lead to decreased drug efficacy. Thus, cancerology can be considered an area of great need to be explored [5].

In recent years, compounds with anticancer activity have been extensively designed and tested as good perspectives by medicinal chemists, such as the possibility of developing promising new prototypes for the treatment of cancer. Previous studies have shown that the pharmacophoric group thiosemicarbazones has several pharmacological properties such, antiparasitic [6, 7, 8, 9], larvicide [10], antimicrobial [11, 12] and antiproliferative [13, 14].

The antitumor activity of thiosemicarbazones seems to be due to an inhibition of DNA synthesis produced by the modification in the reductive conversion of ribonucleotides to deoxyribonucleotides [15]. This biological role is often related with their capability to inhibit the enzyme, ribonucleotide reductase, similar to what is observed with potent anticancer drugs such as triapine [16].

Thiazole nucleus, heterocycle derived from thiosemicarbazone, also have a broad scope in preclinical studies that demonstrate its efficacy in antiparasitic [17, 18, 19], antifungal and antimicrobial [20, 21] and antiproliferative activities [22, 23, 24, 25] in addition to a low cytotoxicity. In previous works, our group identified that 1,3-thiazoles substituted at 2 and 4 positions have potent antitumor and immunomodulatory activity [24, 25]. Regarding the antiproliferative activity, according to Morigi *et al*., thiazole derivatives can act though the inhibition of matrix

metalloproteinases (MMPs), inhibition of kinases and inhibition of anti-apoptotic BCL2 family proteins [26].

On the other hand, it is well known that the presence of C–F bonds in organic molecules has an important effect on properties such as lipophilicity, membrane permeability, pKa, conformation, metabolic stability and pathways, and pharmacokinetic properties [27].

Besides, introducing fluorine atoms and/or fluorine-containing groups into bioactive compounds can improve selectivity, intrinsic potency, increasing efficacy and easiest to administer [28]. Then, more than 20% of the current approved drugs contain at least one fluorine atom [29]. Indeed, compounds containing trifluoromethyl in their structure have shown potent anticancer activity and an increasing number of fluorinated antimitotic/antitumour agents have now becoming available for cancer treatment. [30]. For exemple, trifluoromethylated drugs such as flutamide (I), hydroxyflutamide (II), nilutamide (III) and 5-trifluoromethyl uracil (IV) are widely used for the treatment of metastatic prostate cancer [31].

In this context, and following the work developed by our research group in the development of new antitumor agents [24, 25, 32, 33, 34], the present study aimed to synthesize 1,3-thiazoles derivatives containing 4-(trifluoromethyl)-benzylidene moiety. The prospect inspiration of the new series is presented in **Figure 1**.

PLEASE, INSERT FIGURE 1 HERE

Variations were made in the group linked to position 4 of the thiazole ring, through insertion of aromatic rings with different substitutions in *ortho*, *meta* and *para* positions and the antiproliferative activity against human tumor cell lines of all compounds were evaluated. The structural planning of all series is presented in **Figure 2**.

PLEASE, INSERT FIGURE 2 HERE

Twenty-two derivatives were tested for cytotoxic activity by the MTT assay in five tumor lines. Among the molecules tested, 8 showed significant toxicity in at least three tumor lines tested. The compound **1d** presented better performance and was chosen for studies of the possible mechanism of action.

2 Results and Discussion

2.1 Chemistry

The intermediate thiosemicarbazones **1-3** (R1 = H, Me, Ph, respectively) were prepared in reaction between the 4'-trifluoromethyl-benzaldehyde (commercially obtained) with the corresponding thiosemicarbazide under reflux and catalytic amount of HCl. These intermediate compounds them react with different α-halogenated ketones, obtaining the **1a-3d** series with yields of 22% to 94% (**Scheme 1**). All compounds were identified by infrared (IR) and nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) spectroscopy, mass spectra (ESI-TOF) and their purity was established by elemental analysis (EA).

PLEASE, INSERT SCHEME 1 HERE

NMR data are compatible with the proposed compounds. In theory, two geometrical isomers (*E* and *Z*) about the imine (C=N) double bond are possible for the thiosemicarbazones and the respective thiazoles. However, analysis of the ^{1}H NMR spectra of the compounds indicated one predominant isomer; the *E* isomer by comparison with known analogues [18, 19]. Intramolecular H-bonding involving the proton attached to N4 (in DMSO) with the imine N-atom leads to a distinctive singlet around 12 ppm and this is also seen here [18, 19].

Once thiosemicarbazones were characterized, the respective 1,3-thiazoles were characterized by usual spectroscopy. As exemplified with the ^{1}H NMR analysis of (E)-4-(2,4-dichlorophenyl)-2-(2-(4-(trifluoromethyl) benzylidene) hydrazinyl)thiazole (**1d**), singlet and doublets peaks, corresponding to aromatic protons, were observed at δ 7.5-7.9. For the thiazole ring, a singlet at δ 7.4 was found. NH proton appeared as singlet at δ 12.5. In ^{13}C NMR spectrum of **1d**, peaks of the aromatic carbons were found at δ 126.8-138.3 ppm. The presence of peaks at δ 109.7 and 145.8 ppm, confirm the thiazole cyclisation. Besides, representative ^{1}H-NMR spectrums of some compounds are presented in Supplementary Material.

2.2 Cytotoxic screening of thiosemicarbazones and thiazoles derivatives

A cytotoxic screening against five tumor cells lines in the assay single dose of 25 µg/mL is showed in **Table 1**. Were considered active substances which showed inhibition above 75% in at least two strains of the type adhered (NCI-H292, HEp-2, and HT-29) and a suspension type strain (HL-60 and K562).

Among the 22 substances were tested, 8 showed the above criteria (**1**, **2**, **1b**, **1c**, **1d**, **1e, 1h** e **2b**). Then had IC_{50} values determined after 72 h.

PLEASE, INSERT TABLE 1 HERE

2.3 IC_{50} determination of thiosemicarbazones and thiazoles derivatives

Three thiosemicarbazones derivatives were assessed in tumor cell lines NCI-H292, HEp-2, HT-29, HL-60, and K562. However, only two substances (**1** and **2**) showed cytotoxic activity greater than 75% inhibition in at least two cell lines with adhesive characteristics and a strain in suspension.

Between the three thiosemicarbazones, **1**, **2** and **3** it was observed that the simple addition of phenyl group in the structure reduced the cytotoxicity of the compound **3**. However, **1** (R1 = H) and **2** (R2 = Me) were promising for their antiproliferative action in all tumor lines tested.

1 and **2** showed cytotoxic activity in all tested tumor cell lines. However, **1** showed better activity against NCI-H292, HEp-2, K562 and HL-60, with IC_{50} ranging from 6.27 to 12.74 µM (**Table 3**).

Of the 19 new thiazoles derivatives tested on tumor cell lines, six had significant cytotoxic activity and have determined their IC_{50} (**Table 3**) and only **1c** and **1d** showed significant cytotoxicity to all tested tumor cell lines in the assay single dose of 25 µg/mL (**Table 2**).

Compounds which have a strongly electron withdrawing group (NO_2) in the *para* position (**Table 3**), as observed in the **1b** and **2b**, had a large variation of the IC_{50} values in all tested tumor lines. **1b** showed IC_{50} value ranging from 7.41 to 27.33 µM while the **2b** showed IC_{50} from 11.83 to 47.77 µM. However, although similar, this higher range for **2b** IC_{50} value can be related to the presence of a methyl linked to nitrogen (N-3).

The presence of electron withdrawing group (NO_2) into **1b** showed better results for a NCI-H292 with IC_{50} = 7.41 µM. In contrast, the addition of electrons withdrawing group in the *meta* position (compound **1c**) may be associated with the inhibition of all the cell lines tested in the screening cytotoxic, yet the presence of this group in the *meta* position did not show good results when subjected to tests to determine their IC_{50} values in the tested tumor cell lines, except in the K562 line cell.

The di-substituted compounds 2,4-dichloro (**1d**) and 3,4-dichloro (**1h**) showed excellent cytotoxic activity with IC_{50} less than 10 µM in four tumor cells tested. The variation in the *meta* and *para* position do not appear to interfere with high cytotoxic activity observed.

The **1e** compound showed significant cytotoxicity in three tumor cell lines. The activity of this compound is related to the presence of the methoxy group in the *para* position of the aromatic ring.

PLEASE, INSERT TABLE 2 HERE

From the results obtained, an analysis cytotoxic behavior, over time, of the intermediate compound **1** and the thiazole derivative **1d** were determined from serial dilutions after 24 or 48 h of treatment with K562 cells. The values of IC_{50} are presented in **Table 3**.

PLEASE, INSERT TABLE 3 HERE

The compounds showed low cytotoxicity in K562 cells after 24 hours of treatment. **1** and **1d** were active cytotoxic time-dependent with a maximum effect after 72 h of treatment. From the data obtained, we choose the **1d** to verify the mechanism of action.

2.4 Cytotoxicity in normal cells

The cytotoxicity of the thiosemicarbazones and thiazoles were also evaluated against peripheral blood mononuclear cell (PBMC). The results presented in **Table 4** show that the cytotoxic effects of these compounds were less pronounced in PBMC when compared to tumor cells, with a selectivity index (SI) for myeloblastoid leukaemia

(K562) of 12.49 and 7.85 for **1** and **1d**, respectively. The SI was calculated with the following formula: SI = IC_{50} (PBMC) / IC_{50} (K562).

None of the compounds described here were able to cause hemolysis in human erythrocytes during the study period, even at the highest concentration (250 µg/mL) (**Table 4**). The absence of lytic effects suggests that the cytotoxicity of these compounds is not related to membrane disruption and is likely related to more specific cellular pathways.

PLEASE, INSERT TABLE 4 HERE

2.5 Measurement of mitochondrial membrane potential (ΔΨm)

Several drugs act on mitochondria. Thus, we evaluated by flow cytometry if there is involvement of mitochondria in the mechanism of cell death. The assay was based on incorporation of rhodamine 123 into cells treated with **1d** compound after 48 hours of treatment. The objective was to evaluate the ability of the thiazole derivative to produce changes in mitochondrial transmembrane potential (ΔΨm).

The integrity of mitochondria and cellular bioenergetic functions are controlled by maintaining the mitochondrial membrane potential (ΔΨm). During induction of cell death by drug-induced apoptosis, it is possible to observe mitochondrial alteration, and the event responsible for this is the increase in mitochondrial depolarization (decrease of mitochondrial membrane potential - ΔΨm). This event is related to the initial events that occurred during the apoptosis process [35]. Mitochondrial membrane depolarization is one of the main characteristics of apoptosis-induced cell death [36].

In this study, we evaluated the ability of thiazole derivative **1d** (17 µM and 34 µM) to produce changes in mitochondrial transmembrane potential (ΔΨm) after 48 hours of treatment. Compound **1d** was able to induce mitochondrial depolarization in 25.05% and 37.12% of cells treated with compound **1d** at concentrations of 17 and 34 µM, respectively. The positive control doxorubicin presented depolarization of 23.6% while the negative control presented 12.24% of mitochondrial depolarization (**Figure 3**).

Hsiung and Kadir, [37] observed that mitochondrial depolarization is capable of inducing caspase-3 activation and consequent externalization of phosphatidylserine

(OS) on the plasma membrane of cells treated with substances isolated from plants and thus demonstrated the role of depolarization in apoptosis.

PLEASE, INSERT FIGURE 3 HERE

Recent studies have demonstrated that thiazol-derived compounds are capable of inducing mitochondrial membrane depolarization and consequent apoptosis in human acute myeloid leukemia U937 cells [38]. Similarly, benzothiazole YLT322 was able to depolarize the mitochondrial potential and trigger the release of cytochrome c from the mitochondria to the cytosol showing the involvement of this derivative in the intrinsic pathway of apoptosis [39].

Kamal *et al.* [40] also observed that a benzothiazole linked to phenyl pyridopyrimidinones was able to induce apoptosis by depolarizing the mitochondrial membrane of cervical cancer cells ME-180 and activate caspase-3 in cervical cancer cell line ME-180.

Our results suggest that compound **1d** is capable of inducing cell death with mitochondrial involvement in the two tested doses. However, complementary studies evaluating the involvement of caspases and cytochrome c must be performed to elucidate the involvement of mitochondria on the induction of apoptosis in K562 cells from compound **1d**.

2.6 Cell cycle assay

The cell cycle is related to a series of events responsible for cell division and duplication. The cell cycle presents four distinct phases: G1, S (synthesis), G2 (interphase) and M (mitosis) phases. The harnessing of energy and material for DNA synthesis occurs in the G1 phase. Then the cell replicates its DNA in the S phase. The G2 phase prepares the cell for the mitosis itself where division of the nucleus and cytoplasm of the cell takes place [41]. Due to the importance of the cell cycle in the process of tumor progression we evaluated if cell growth inhibition in K562 occurred due to cell cycle arrest by flow cytometry 48 hours after treatment.

Many antitumor treatments have better results when cells are in the process of cell division. The explanation is because tumor cells show loss of control of differentiation and alteration in the cell cycle [42]. Due to the importance of this event

in the process of tumor progression we evaluated the action of compound **1d** on the cell cycle by flow cytometry.

In this study K562 cells were treated with these compounds at concentrations of 17 and 34 μM for 48 h. The data obtained clearly indicated that these compounds show G1 cell cycle arrest in comparison with the untreated cells. The compound **1d** (17 and 34 μM) showed 85.25 and 82.74 % of cells in G1 phase (**Figure 4**). The positive control doxorubicin had cell cycle arrest in G2/M.

Several thiazole derivatives have been described in the literature as stop inducers in different phases of the G0 / G1, S and G2 / M cell cycle and in different tumor cell lines [41, 42, 43, 44, 45].

Senviki *et al.* [42] observed that treatment with (5- [5- (2-Hydroxyphenyl) -3-phenyl-4,5-dihydropyrazol-1-ylmethylene] -3- (3-acetoxyphenyl) -2-thioxothiazolidin-4-one) after 48 hours of treatment in leukemic cells HL-60 was also able to induce cell cycle arrest in the G0 / G1 phase.

PLEASE, INSERT FIGURE 4 HERE

2.7 DNA fragmentation assay

An important feature in the process of cell death by apoptosis is the presence of fragmented DNA. However, it is also possible to observe other changes in nuclear morphology such as nuclear condensation, chromosomal DNA cleavage, formation of membrane blebs and presence of apoptotic bodies [46].

In order to evaluate DNA fragmentation, we used the same principle of cell cycle analysis from the incorporation of propidium iodide in the cells. Cells with fragmented DNA emit less fluorescence by incorporating less propidium iodide than a cell with whole DNA. For the tumor line tested, compound **1d** induced DNA fragmentation at the two concentrations analyzed and obtained maximum effect at the concentration of 34 μM which represented 22.43% of the cells presented fragmented DNA. Compound **1d** at the concentration of 17 μM presented 19.74% of the cells with fragmented DNA (**Figure 5**).

PLEASE, INSERT FIGURE 5 HERE

Compound **1d** disrupted the K562-type leukemic cell cycle in G1 phase and was able to depolarize the mitochondrial membrane. Altogether, these data and the presence of cells with fragmented DNA may infer that the probable mechanism of cell death occurs by apoptosis. However, subsequent studies related to this cell death pathway should be performed.

2.8 Physicochemical and ADME parameters

We evaluated if the synthesized compounds physicochemical properties are within the Lipinski's Rule of Five, which are important for pharmacokinetics and drug development. For this purpose, physicochemical and ADME properties were calculated using the SwissADME (a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules). Compound obeying at least three of the four criteria are considered to adhere to Lipinski Rule [47]. All synthetized compounds are compatible with Lipinski Rule. Another interesting property is the number of rotatable bonds and the polar surface area (PSA). A large number of rotatable bonds (≥10) has been associated with poor oral bioavailability [48]. Compounds with a low PSA (≤ 140 $Å^2$) tend to have higher oral bioavailability [48, 49]. All synthetized compounds have appropriate PSA and number of rotatable bonds. These data are presented in the supplementary material.

As demonstrated in **Table 5**, the most active compounds shown variable permeability based on gastrointestinal absorption (GI), according to the BOILED-Egg predictive model (Brain Or IntestinaL EstimateD permeation method). Three compounds showed high gastrointestinal absorption (**1**, **2** and **1e**). With respect to oral bioavailability, it's expected 0.55 of probability of oral bioavailability score > 10% in the rat for all compounds, greater than doxorubicin (0.17). All these data, suggests the *in silico* good druglikeness profile and great chemical stabilities for this thiazole compounds.

PLEASE, INSERT TABLE 5 HERE

3 Conclusion

A series of three thiosemicarbazones and nineteen thiazoles derivatives that insert a 4-(trifluoromethyl)-benzylidene moiety were synthesized. Between them, 8 compounds

were identified with significant cytotoxic activity in at least three tumor cell lines. Their IC_{50} were determined and their action on red blood cells and PBMC were also tested. Compound **1d** presented as a promising antitumor candidate and had its mechanism of action evaluated by flow cytometry. Our results showed cell cycle arrest in G1 phase, DNA fragmentation and alteration in mitochondrial membrane potential by compound **1d** for the two concentrations tested. These data suggest a process of cell death, at least in part, by apoptosis, with involvement of mitochondrial pathway in the mechanism of action. Complementary studies, such as involvement of caspases and cytochrome c should be performed to better elucidate the induction of cell death produced by **1d**. The physicochemical and ADME properties of synthesized compounds were calculated *in silico* and the data suggests thiazole compounds fits druglikeness profile. Thus, our results show that thiazole derivatives can be used in the development of new drugs with anticancer activity.

4 Experimental Section

4.1 Chemistry

The reagents were purchased from Acros Organics, Fluka, Sigma-Aldrich and Vetec or Dynamics solvents. Deuterated solvents (DMSO-d 6, $CDCl_3$, D_2O) were of the trademark CIL (Tedia Brazil). The reactions were monitored by thin layer chromatography using silica gel 60, containing fluorescent indicator F254. Chromatographic plates were visualized under UV light (with double wavelength 365 or 254 nm). Melting points were measured using a Thomas Hoover capillary and values (°C) were reported thereafter. For all of the novel compounds, ^{1}H and ^{13}C NMR analyzes and, where necessary, two-dimensional analysis (DEPT) as well as the addition of deuterated water to localize NH signals were performed. All compounds were solubilized in DMSO-d_6. ^{1}H and ^{13}C NMR spectra were obtained using Unity Plus model Variant instruments (400 MHz to ^{1}H, 100 MHz to ^{13}C) or Bruker AMX (300 MHz to ^{1}H, 75.5 MHz to ^{13}C) using tetramethylsilane (TMS) as internal standard. The number of signals in ^{1}H NMR spectra was designated as follows: s/singlet; d/doublet, t/triplet, dd/double doublet, q/quartet, m/multiplet, and the coupling constants, in hertz, as *J*. Infrared spectroscopy was performed on a Bruker instrument (IFS 66 model) using KBr.

4.1.1 General Procedure for synthesis of compounds 1, 2 and 3

The compounds **1**, **2** and **3** was prepared by reacting commercially available respective thiosemicarbazide with 4-(Trifluoromethyl)benzaldehyde (1:1 mol ratio) using ethanol under reflux in the presence of catalytic amount of hydrochloric acid, for 3 hours. Finally, water was added at the end of the reaction for precipitation of the compounds. This reaction condition led to yield between 85% to 94%. The reaction was monitored by thin layer chromatography (TLC). The resulting solid was filtered through a sintered funnel and recrystallized from toluene to give the pure product.

4.1.1.1 (E)-2-[4-(trifluoromethyl)benzylidene]hydrazinecarbothioamide (1)

White crystals; Yield 85%; m.p.(ºC) 166-170; Rf:0,41 (hexane / ethyl acetate 7:3). IR (KBr, cm^{-1}): 3272.63 (NH), 1698.36 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 3.350 (s, 2H, NH_2), 7.673 (d, *J* = 7.8 Hz, 2H, Ar), 7.804 (d, *J* = 8.1 Hz, 2H, Ar), 8.301 (s, 1H, HC=N), 11.598 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 124.000(CF_3) 125.278 (C, Ar), 125.324 (C, Ar), 127.651 (C, Ar), 129.971 (C, Ar), 134.488 (C, Ar), 138.147 (C, Ar), 140.219 (C=N), 178.297 (C=S). Anal. Calcd for $C_9H_8F_3N_3S$: C, 43.72; H, 3.26; N, 17.00; S, 12.97. found: C, 43.94; H, 3.04; N, 15.02; S, 11.89. HRMS: 343.9749 [M+H] +

4.1.1.2 (E)-N-methyl-2-[4-(trifluoromethyl)benzylidene] hydrazinecarbothioamide (2)

White crystals; Yield 94%; m.p.(ºC) 234-236; Rf:0,75 (hexane / ethyl acetate 7:3). IR (KBr, cm^{-1}): 3158.76 (NH), 1539.93 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 3.02 (d, *J* = 4.2 Hz, 3H, CH_3), 7.756 (d, *J* = 7.8 Hz, 2H, Ar), 8.025 (d, *J* = 7.5 Hz, 2H, Ar), 8.687 (s, 1H, HC=N), 11.690 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 30.923 (CH_3), 125.134(CF_3), 125.522 (C, Ar), 127.727 (C, Ar), 138.372 (C, Ar), 138.784(C=N), 177.915 (C=S). Anal. Calcd for $C_{10}H_{10}F_3N_3S$: C, 45.97; H, 3.86; N, 16.08; S, 12.27. found: C, 46.22; H, 2.77; N, 15.81; S, 11.13. HRMS: 262.0527 [M+H] +

4.1.1.3 (E)-N-phenyl-2-[4-(trifluoromethyl)benzylidene] hydrazinecarbothioamide (3)

White crystals ; Yield 93%; m.p.(ºC) 190-193; Rf:0,65 (hexane / ethyl acetate 7:3). IR (KBr, cm^{-1}): 3336.09(NH), 1541.11 (HC=N). ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm:

7.227 (t, *J* = 7.4 Hz, 1H, Ar), 7.385 (t, *J* = 7.8 Hz, 2H, Ar), 7.544 (d, *J* = 8.4 Hz, 2H, Ar), 7.767 (d *J* = 8 Hz, 2H, Ar), 8.138 (d, *J* = 8.4 Hz, 2H, Ar), 8.213 (s, 1H, HC=N), 10.252 (s, 1H, NH), 11.986 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 123.2 (C, Ar), 125.9 (CF_3), 125.917(C, Ar), 126.0 (C, Ar), 126.6 (C, Ar), 128.5 (C, Ar), 128.6 (C, Ar), 129.8 (C, Ar), 130.1 (C, Ar), 138,5 (C, Ar), 139.4 (C, Ar), 141.4 (C=N), 176.9 (C=S). Anal. Calcd for $C_{15}H_{12}F_3N_3S$: C, 55.72; H, 3.74; N, 13.00; S, 9.92. found: C, 56.12; H,2.91; N, 12.82; S, 8.06. HRMS: 324.0704 [M+H] +

4.1.2 General procedure for synthesis of the series (1a-3d)

In a round bottom flask, arylthiosemicarbazone (**1**, **2** or **3**) and their acetophenone in 2-propanol (20 ml). The reaction mixture was kept at room temperature for 1 h. Reactions were monitored by thin layer chromatography (TLC). The resulting solid was filtered through sintered funnel with distilled water, yielding the pure product.

4.1.2.1 (E)-4-phenyl-2-{2-[4-(trifluoromethyl)benzylidene]hydrazinyl}thiazole (1a)

Light yellow crystals ; Yield 47%; m.p.(ºC) 212-215; Rf: 0,72 (hexane / ethyl acetate 7:3). IR (KBr, cm^{-1}): 3413.82(NH), 1634.54 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 12.5(s, 1H, NH), 8.112 (s, 1H, HC=N), 7.857 (d, *J* = 9,6 Hz 4H, CH Ar), 7.778 (d, *J* = 10.8 Hz 2H, CH Ar), 7.410 (m, 3H, CH Ar), 7.304 (t, *J* = 6.5 Hz 1H, CH Ar); ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 167.9 (S-C), 150.3 (C thiazole),139.6 (C=N), 138.4 (C, Ar), 134.4 (C, Ar), 129.1(C, Ar), 128.7(C, Ar), 127.7 (C, Ar), 126.8 (C, Ar), 125.8 (C, Ar), 125.8 (C, Ar), 125.6 (CF_3) e 104.2 (C thiazole). Anal. Calcd for $C_{17}H_{12}F_3N_3S$: C, 58.78; H, 3.48; N, 12.1 ; S, 9.23. found: C,54.25; H, 2.23; N, 10.15; S, 7.13. HRMS: 348,0710 [M+H] +

4.1.2.2 (E)-4-(4-nitrophenyl)-2-{2-[4-(trifluoromethyl)benzylidene]hydrazinyl} thiazole (1b)

Light yellow crystals ; Yield 34%; m.p.(ºC) 226-230; Rf: 0,66 (hexane / ethyl acetate 7:3). IR (KBr, cm^{-1}): 3298.22(NH), 1594.12 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 8.273 (d, *J* = 11,6 Hz 2H, CH Ar), 8.109 (m, 3H, CH Ar), 7.862 (d, *J* = 10.8 Hz 2H, CH Ar), 7.781 (m, 3H, CH Ar), 4.264 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 168.4 (S-C), 148.6 (C thiazole), 146.2 (C, Ar), 140.5 (C=N), 139.9 (C, Ar), 138.2 (C, Ar), 130.2 (C, Ar), 129.2 (C, Ar), 126.8 (C, Ar), 126.4 (C,

Ar), 125.8 (CF3), 124.2 (C, Ar), 123.9 (C, Ar), 109.1 (C thiazole). Anal. Calcd for $C_{17}H_{11}F_3N_4O_2S$: C, 52.04; H, 2.83; N, 14.28; S, 8.17. found: C, 53.51; H, 1.81; N, 12.76; S, 5.34. HRMS: 393,0565 [M+H] +

4.1.2.3 (E)-4-(3-nitrophenyl)-2-{2-[4-(trifluoromethyl)benzylidene]hydrazinyl} thiazole (1c)

Light yellow crystals ; Yield 63%; m.p.(ºC) 229-231; Rf: 0,61 (hexane / ethyl acetate 7:3). IR (KBr, cm^{-1}): 3049.09(NH), 1618.99 (C=N);^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 8.658 (s, 1H, CH Ar),8.293 (m, 2H, CH Ar), 8.097 (s, 1H, C=N), 7.783 (m, 6H, CH Ar), 4.295 (s, 1H, NH);^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 168.7 (S-C), 167.3 (C-NO_2), 148.7 (C=N), 140.2 (C4 thiazole), 138.7 (C, Ar), 136.5 (C, Ar), 132.0 (C, Ar), 130.7 (C, Ar), 127.3 C, Ar), 126.2(C, Ar), 122.6 (C, Ar), 120.4 (CF_3), 116.5 (C, Ar), 117.2 (C,Ar), 107.4 (C thiazole). Anal. Calcd for $C_{17}H_{11}F_3N_4O_2S$: C, 52.04; H, 2.83; N, 14.28; S, 8.17. found: C, 51.36; H, 1.51; N, 11.01; S, 8.86. HRMS: 393,0565 [M+H] +

4.1.2.4 (E)-4-(2,4-dichlorophenyl)-2-{2-[4-(trifluoromethyl)benzylidene] hydrazinyl}thiazole (1d)

White crystals; Yield 50%; m.p.(ºC) 165-168; Rf: 0,80 (hexane / ethyl acetate 7:3). IR (KBr, cm^{-1}): 1623.63 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 12.462 (s, 1H, NH), 8.128 (s,1H,HC=N), 7.9 (s,1H, CH Ar), 7.862 (d, *J* = 10 Hz 2H, CH Ar), 7.776 (d, *J* = 8 Hz 2H, CH Ar), 7.691 (s, 1H, CH Ar), 7.505 (d, *J* = 8,4 Hz 2H, CH Ar), 7.443 (s, 1H, CH thiazole);^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 167.2 (S-C), 145.8 (C thiazole),139.7 (C=N), 138.3 (C, Ar), 132.6 (C-Cl), 132.2 (C-Cl), 131.9(C, Ar), 131.6(C, Ar), 129.8 (C, Ar), 129.0 (C, Ar), 128.7 (C, Ar), 127.5 (C, Ar), 126.8 (C, Ar), 125.7 (CF_3), 109.7 (C thiazole). Anal. Calcd for $C_{17}H_{10}Cl_2F_3N_3S$: C, 49.06; H, 2.42; N, 10.1; S, 7.7. found: C, 45.71; H, 2.10; N, 7.10; S, 6.30. HRMS: 415,9950 [M+H] +

4.1.2.5 (E)-4-(4-methoxyphenyl)-2-{2-[4-(trifluoromethyl)benzylidene] hydrazinyl}thiazole (1e)

Yellow crystals; Yield 88%; m.p.(ºC) 210-213; Rf: 0,64 (hexane / ethyl acetate 7:3). IR (KBr, cm^{-1}): 3421.75 (NH); 1611.53 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 12.500 (s, 1H, NH), 8.099 (s,1H,HC=N), 7.858 (d, *J* = 8 Hz 2H, CH Ar),7.783

(d, *J* = 7,2 Hz 4H, CH Ar), 7.203 (s, 1H,CH thiazole), 6.971 (d, *J* = 8.4 Hz 2H, CH Ar) e 3.782 (s, 3H, CH3); ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 167.2 (S-C), 145.8 (C thiazole),139.7 (C=N), 138.3 (C, Ar), 132.6 (C-Cl), 132.2 (C-Cl), 131.9(C, Ar), 131.6(C, Ar), 129.8 (C, Ar), 129.0 (C, Ar), 128.7 (C, Ar), 127.5 (C, Ar), 126.8 (C, Ar), 125.7 (CF_3), 109.7 (C thiazole). Anal. Calcd for $C_{18}H_{14}F_3N_3OS$: C, 57.29; H, 3.74; N, 11.13; S, 8.5. found: C, 54.93; H, 2.90; N, 9.39; S, 6.77. HRMS: 378,0814 [M+H] +

4.1.2.6 (E)-4-(4-chlorophenyl)-2-{2-[4-(trifluoromethyl)benzylidene]hydrazinyl} thiazole (1f)

Light Yellow crystals; Yield 90%; m.p.(ºC) 240-243; Rf: 0,74 (hexane / ethyl acetate 7:3). IR (KBr, cm^{-1}): 3451.89 (NH); 1623.25 (C=N);^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 12.441 (s, 1H, NH), 8.111 (s, 1H,HC=N), 7.861(m ,4H, CH Ar), 7.775 (d, *J* = 7,2Hz 2H, CH Ar), 7.598 (d, ,*J* = 10.4 Hz 3H, CH Ar);^{13}C NMR (100 MHz, DMSO-d6), δ ppm: 168.1 (S-C), 149.2 (C thiazole), 139.7 (C=N), 138.3 (C, Ar), 133.3 (C, Ar), 132.0 (C-Cl), 129.5 (C, Ar), 128.6 (C, Ar), 127.3 (C, Ar), 126.8 (C, Ar), 125.7 (C,F_3), 124.7 (C, Ar) 105 (C thiazole). Anal. Calcd for $C_{17}H_{11}ClF_3N_3S$: C, 53.48; H, 2.9; N, 11.01; S, 8.4. found: C, 52.34; H, 2.22; N, 8.83; S, 7.84. HRMS: 382,0342 [M+H] +

4.1.2.7 (E)-4-(4-bromophenyl)-2-{2-[4-(trifluoromethyl)benzylidene]hydrazinyl} thiazole (1g)

Yellow crystals; Yield 68%; m.p.(ºC) 235-238; Rf: 0,71 (hexane / ethyl acetate 7:3). IR (KBr, cm^{-1}): 3411.28 (NH), 1618.80(C=N); ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 12.501 (s, 1H, NH), 7,820 (m, 6H, CH Ar), 7.602 (d, *J* = 8,4 Hz 2H, CH Ar), 7.462 (s, 1H, CH thiazole); ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 168.1 (S-C), 149.3 (C thiazole), 139.6 (C=N), 138.3 (C, Ar), 133.7 (C, Ar), 131.6 (C, Ar), 127.5 (C, Ar), 126.7 (C, Ar), 125.9 (C,Ar) 125.7 (CF3), 120.6(C-Br) e 105.1 (C thiazole). Anal. Calcd for $C_{17}H_{11}BrF_3N_3S$: C, 47.9; H, 2.60; N, 9.86; S, 7.52. found: C, 43.17; H, 1.98; N, 7.49; S, 6.15. HRMS: 427,9948 [M+H] +

4.1.2.8 (E)-4-(3,4-dichlorophenyl)-2-{2-[4-(trifluoromethyl)benzylidene] hydrazinyl}thiazole (1h)

Beige crystals; Yield 47%; m.p.(ºC) 236-238; Rf: 0,76 (hexane/ethyl acetate 7:3). IR (KBr, cm^{-1}): 3411.24 (NH), 1610.73 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 12.452 (s, 1H, NH), 8.1 (s, 1H, HC=N), 7.800 (m ,6H, CH Ar), 7.651 (m, 1H, CH Ar), 7.587 (s, 1H, CH thiazole); ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 168.1 (S-C), 147.9 (C thiazole),139.8 (C=N), 138.2 (C, Ar), 135.0 (C-Cl), 131.4 (C-Cl), 130.9 (C, Ar), 129.8 (C, Ar), 127.7 (C, Ar), 127.160 (C, Ar), 126.7 (C, Ar), 125.6 (C, Ar), 124.5 (CF_3), 122.8 (C, Ar) e 106.4 (C, thiazole). Anal. Calcd for $C_{17}H_{10}Cl_2F_3N_3S$: C, 49.06; H, 2.42; N, 10.1; S, 7.7. found: C, 41.27; H, 1.00; N, 8.23; S, 5.62. HRMS: 414,9924 [M+H] +

4.1.2.9 (E)-5-methyl-4-phenyl-2-{2-[4-(trifluoromethyl)benzylidene]hydrazinyl} thiazole (1i)

Yellow crystals; Yield 84%; m.p.(ºC) 226-229; Rf: 0,60 (hexane/ethyl acetate 7:3). IR (KBr, cm^{-1}): 3366.11 (NH), 1616.63 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 12.441 (s, 1H, NH), 8.142 (s, 1H, HC=N) 7.877(d, *J*= 7,6 Hz 2H, CH Ar), 7.784 (d, *J* = 7,6 Hz 2H, CH Ar), 7.607 (d, *J* = 7,6 Hz 2H, CH Ar), 7.462 (t, *J* = 7,4 Hz 2H, CH Ar), 7.378 (m, 1H, CH Ar), 2.408 (s, 1H, CH3); ^{13}C NMR (100 MHz, DMSO-d_6), δ ppm: 164.4 (S-C), 146.1 (C thiazole), 142.5 (C=N), 138.2 (C, Ar), 133.5 (C, Ar), 132.3 (C, Ar), 128.4 (C, Ar), 128.1 (C, Ar), 127.7 (C, Ar), 126.9 (C, Ar), 125.7 (C, Ar), 124.7 (CF3), 117.4 (C thiazole), 12.1 (CH3). Anal. Calcd for $C_{18}H_{14}F_3N_3S$: C, 59.82; H, 3.90; N, 15.77; S, 8.87. found: C, 57.87 H, 2.83; N, 9.32; S, 6.63. HRMS: 362,0856 [M+H] +

4.1.2.10 (E)-4-(naphthalen-1-yl)-2-{2-[4-(trifluoromethyl)benzylidene] hydrazinyl}thiazole (1j)

Yellow crystals ; Yield 76%; m.p.(ºC) 217-220; Rf: 0,71 (hexane/ethyl acetate 7:3). IR (KBr, cm^{-1}3421.17 (NH), 1613.46 (C=N); ^{1}H NMR (400 MHz, DMSO-d6), δ ppm: 12.456 (s, 1H, NH), 8.402 (s, 1H, CH Ar), 8.156 (s ,1H, C=N), 8.026 (d, *J* = 8,4 Hz 1H, CH Ar), 7.924 (m, 6H, CH Ar), 7.800(d, *J* = 7,6 Hz 2H, CH Ar), 7.541 (d, 2H, CH Ar); ^{13}C NMR (100 MHz, DMSO-d6), δ ppm: 168.0 (S-C), 150.3 (C thiazole), 139.6 (C=N), 138.3 (C, Ar), 133.1 (C, Ar), 132.5 (C, Ar), 131.9 (C, Ar), 128.7 (CF3), 128.1 (C, Ar), 127.8 (C, Ar), 126.7 (C, Ar), 126.4 (C, Ar), 126.1 (C, Ar), 125.7 (C, Ar), 125.5 (C, Ar), 124.1 (C, Ar), 123.9 (C, Ar), 105.0 (C thiazole). Anal.

Calcd for $C_{21}H_{14}F_3N_3S$: C, 63.47; H, 3.55; N, 10.57; S, 8.07. found: C, 60.08 H, 2.85; N, 9.60; S, 7.17. HRMS: 398,0866 [M+H] +

4.1.2.11 (E)-4-(p-tolyl)-2-{2-[4-(trifluoromethyl)benzylidene]hydrazinyl}thiazole (1k)

Yellow crystals ; Yield 76%; m.p.(ºC) 217-220; Rf: 0,71 (hexane/ethyl acetate 7:3). IR (KBr, cm^{-1}): 3421.17 (NH), 1613.46 (C=N); ^{1}H NMR (400 MHz, DMSO-d6), δ ppm: 12.418 (s, 1H, NH), 8.109 (s, 1H, C=N), 7.856 (d, *J* = 7,6 Hz 2H, C Ar), 7.756 (m, 4H, C Ar), 7.291 (s, 1H, CH thiazole), 7.212 (d, *J* = 7,6 Hz 2H, CH Ar), 2.496 (s, 3H, CH3); ^{13}C NMR (100 MHz, DMSO-d6), δ ppm: 167.8 (S-C), 150.3 (C thiazole), 143.3 (C=N), 139.6 (C, Ar), 138.3 (C, Ar), 136.9 (C, Ar), 131.7 (C, Ar), 129.2 (C, Ar), 128.9 (C, Ar), 127.1 (C,Ar), 126.7 (C, Ar), 125.6 (CF3), 103.3 (C thiazole), 20.8 (CH3). Anal. Calcd for $C_{18}H_{14}F_3N_3S$: C, 59.82; H, 3.90; N, 11.63; S, 8.87. found: C, 56.56 H, 2.73; N, 9.82; S, 6.15. HRMS: 362,0864 [M+H] +

4.1.2.12 (E)-4-(4-fluorophenyl)-2-{2-[4-(trifluoromethyl)benzylidene]hydrazinyl} thiazole (1l)

Yellow crystals ; Yield 22%; m.p.(ºC) 212-215; Rf: 0,71 (hexane/ethyl acetate 7:3). IR (KBr, cm^{-1}): 3326.72 (NH), 1611.91 (C=N); ^{1}H NMR (400 MHz, DMSO-d6), δ ppm: 12.391 (s, 1H, NH), 8.114 s, 1H, C=N), 8.035(d, *J* = 7,2 Hz 2H, CH Ar), 7.871 (m, 5H, CH Ar), 7.753 (m, 4H, C Ar), 7.361 (s, 1H, CH thiazole), 7.251 (d, *J* = 7,6 Hz 2H, CH Ar); ^{13}C NMR (100 MHz, DMSO-d6), δ ppm: 178.3 (S-C, 168.0 (C-F), 150.3 (C thiazole), 140.3 (C=N), 139.5 (C, Ar), 138.3 (C, Ar), 127.8 (C, Ar), 127.6 (C, Ar), 127.5 (C, Ar), 126.5 (C, Ar), 126.7 (C, Ar), 125.6 (CF3), 115.5 (C, Ar) e 108.9 (C thiazole). Anal. Calcd for $C_{17}H_{11}F_4N_3S$: C, 55.89; H, 3.03; N, 11.5; S, 8.78. found: C, 49.67, H, 2.82; N, 10.68; S, 5.66. HRMS: 366.0610 [M+H] +

4.1.2.13 (E)-4-(4-methoxyphenyl)-3-methyl-2-{(E)-[4-(trifluoromethyl) benzylidene]hydrazono}-2,3-dihydrothiazole (2a)

Yellow crystals ; Yield 72%; m.p.(ºC) 166-168; Rf: 0,71 (hexane/ethyl acetate 7:3). IR (KBr, cm^{-1}): 1590.69 (C=N); ^{1}H NMR (400 MHz, DMSO-d6), δ ppm: 8.559 (s, 1H, HC=N), 7.968 (d, *J* = 10 Hz 2H, 2H, CH Ar), 7.834 (d, *J* = 10,8 Hz 2H, 2H, CH Ar), 7.481 (d, *J* = 10 Hz 2H, 2H, CH Ar),7.090 (d, *J* = 10 Hz 2H, 2H, CH Ar), 6.865 (s, 1H, CH thiazole), 3.824 (s, 3H, O-CH3), 3.471 (s, 3H, CH3); ^{13}C NMR (100 MHz,

DMSO-d6), δ ppm: 169.5 (C=N), 160.3 (C, Ar), 150.4 (C-S), 148.7 (C thiazole), 141.7 (C=N), 137.8 (C, Ar), 130.8 (C, Ar), 127.7 (C, Ar), 125.9 (C, Ar), 124.5 (CF_3)121.2 (C, Ar), 114.4 (C, Ar), 103.8 (C, thiazole), 55.4 (O-CH3) e 34.9 (CH3). . Calcd for $C_{19}H_{16}F_3N_3OS$: 58.30; H, 4.12; N, 10.74; S, 8.19. found: C, 56.91, H, 2.70; N, 8.60; S, 6.30. HRMS: 392,1009 [M+H] +

4.1.2.14 (E)-3-methyl-4-(4-nitrophenyl)-2-{[(E)-4-(trifluoromethyl)benzylidene] hydrazono}-2,3-dihydrothiazole (2b)

White crystals ; Yield 83%; m.p.(ºC) 235-237; Rf: 0,66 (hexane/ethyl acetate 7:3). IR (KBr, cm^{-1}): 1608.64 (C=N); ^{1}H NMR (400 MHz, DMSO-d6), δ ppm: 8.513 (s, 1H, HC=N), 8.352 (d, *J* = 11,2 Hz 2H, CH Ar), 7.953 (d, *J* = 10,4 Hz 2H, CH Ar), 7.836 (t, *J* = 13,2 Hz 3H, CH Ar), 7.023 (s, 1H, CH thiazole), 6.865 (s, 1H, CH thiazole), 3.462 (s, 3H, CH3); ^{13}C NMR (100 MHz, DMSO-d6), δ ppm: 170.7 (C=N), 149.6 (HC=N), 148.2 (C-NO_2), 139.9 (C, thiazole), 138.7 (C, Ar), 136.2 (C, Ar), 130.7 (C, Ar), 128.1 (C, Ar), 127.2 (C, Ar), 126.3 (C, Ar), 125.4 (C, Ar), 124.4 (CF_3), 123.3 (C, Ar), 106.3 (C, thiazole), 35.2 (CH3). Anal. Calcd for $C_{18}H_{13}F_3N_4O_2S$: C, 53.20; H, 3.22; N, 13.79; S, 7.89. found: C, 54.04, H, 2.31; N, 11.19; S,6.12. HRMS: 407,0711 [M+H] +

4.1.2.15 (E)-4-(2,4-dichlorophenyl)-3-methyl-2-{(E)-[4-(trifluoromethyl) benzylidene]hydrazono}-2,3-dihydrothiazole (2c)

Yellow crystals ; Yield 70%; m.p.(ºC) 201-204; Rf: 0,65 (hexane/ethyl acetate 7:3). IR (KBr, cm^{-1}): 1540.96 (C=N); ^{1}H NMR (400 MHz, DMSO-d6), δ ppm: 8.400 (s, 1H, HC=N), 7.786 (d, *J* = 10 Hz 2H, CH Ar), 7.687 (d, *J* = 8 Hz 2H, CH Ar), 7.480 (s, 1H, CH Ar), 7.290 (m, 2H, CH Ar), 6.777 (s, 1H, CH thiazole), 3.335(s, 3H, CH3); ^{13}C NMR (100 MHz, DMSO-d6), δ ppm: 167.2 (S-C), 149.4 (C=N), 144.98 (C, Ar),), 139.0 (C thiazole), 138.7 (C, Ar132.7 (C,Ar), 132.4 (C,Ar), 129.7 (C, Ar), 129.5 (C, Ar), 128.9 (C, Ar), 126.8 (C, Ar), 125.3 (C, Ar), 125.2 (C, Ar), 124.1 (CF3), 108.7 (C thiazole), 30.85 (CH_3). Anal. Calcd for $C_{18}H_{12}Cl_2F_3N_3S$: C, 50.25; H, 2.81; N, 9.77; S, 7.45. found: C, 47.68, H, 2.43; N, 8.09; S, 4.12. HRMS: 430.9950 [M+H] +

4.1.2.16 (E)-4-(2,4-dichlorophenyl)-3-phenyl-2-{(E)-[4-(trifluoromethyl) benzylidene]hydrazono}-2,3-dihydrothiazole (3a)

White crystals ; Yield 84%; m.p.(ºC) 2055-208; Rf: 0,79 (hexane/ethyl acetate 7:3). IR (KBr, cm^{-1}): 1540.96 (C=N); ^{1}H NMR (400 MHz, DMSO-d6), δ ppm: 8.217 (s, 1H, HC=N), 8.140 (d, *J* = 8 Hz 2H, CH Ar), 7.776 (d, *J* = 8 Hz 2H, CH Ar), 7.552 (d, *J* = 7.2 Hz 3H, CH Ar), 7.384 (m, 4H, CH Ar), 7.227 (t, *J* = 4.8 Hz 1H, CH Ar), 6.630 (s, 1H, CH thiazole); ^{13}C NMR (100 MHz, DMSO-d6), δ ppm: 158.3 (S-C), 149.4 (C=N), 147.3 (C, thiazole) 141.2 (C, Ar), 139.0 (C, Ar), 133.3 (C, Ar), 133,4 (C, Ar), 132.5 (C-Cl), 129.7 (C, Ar), 129. 6 (C, Ar), 129.5 (C, Ar), 129.4 (C, Ar), 129.3 (C, Ar), 129.2 (C, Ar), 128.9 (C, Ar), 126.8 (C, Ar), 125.2 (C-Cl), 125.1 (C, Ar), 124.1 (CF3), 122.8 (C, Ar), 122.6 (C, Ar), 122.4 (C, Ar), 106.3 (C, thiazole). Anal. Calcd for $C_{23}H_{14}Cl_2F_3N_3S$: C, 56.11; H, 2.87; N, 8.53; S, 6.51. found: C, 55.56, H, 3.79; N, 12.89; S, 5.97. HRMS: 492.0237 [M+H] +

4.1.2.17 (E)-4-(4-methoxyphenyl)-3-phenyl-2-{(E)-[4-(trifluoromethyl) benzylidene]hydrazono}-2,3-dihydrothiazole (3b)

White crystals ; Yield 75%; m.p.(ºC) 197-200; Rf: 0,71 (hexane/ethyl acetate 7:3). IR (KBr, cm^{-1}): 1589.86 (C=N); ^{1}H NMR (400 MHz, DMSO-d6), δ ppm: 8.274 (s, 1H, HC=N), 7.882 (d, *J* = 8.4 Hz 2H, CH Ar), 7.773 (d, *J* = 8.4 Hz 2H, CH Ar), 7.362 (m, 5H, CH Ar), 7.198 (d, *J* = 8.8 Hz 2H, CH Ar), 7.099 (d, *J* = 8.8 Hz 2H, CH Ar), 6.818 (m, 1H, CH Ar), 6.719 (s, 1H, CH thiazole), 3.697 (s, 3H, CH3); ^{13}C NMR (100 MHz, DMSO-d6), δ ppm: 171.1 (C-O), 159.3 (S-C), 149.6 (C=N), 147.3 (C, Ar) 140.0 (C, Ar), 138.6 (C, Ar), 137.3 (C, Ar), 136.8 (C, Ar), 129.9 (C, Ar), 129.1 (C, Ar), 128.8 (C, Ar), 128.7 (C, Ar), 128.5 (C, Ar), 127.5 (C, Ar), 125.6 (C, Ar), 125.5 (C, Ar), 122.3 (C, Ar), 113.6 (C, Ar), 55.1 (C thiazole). Anal. Calcd for $C_{24}H_{18}F_3N_3OS$: C, 63.57; H, 4; N, 9.27; S, 7.07. found: C, 62.78, H, 3.07; N, 7.53; S, 7.07. HRMS: 453.1122 [M+H] +

4.1.2.18 (E)-4-(4-chlorophenyl)-3-phenyl-2-{(E)-[4-(trifluoromethyl)benzylidene] hydrazono}-2,3-dihydrothiazole (3c)

White crystals ; Yield 68%; m.p.(ºC) 198-200; Rf: 0,73 (hexane/ethyl acetate 7:3). IR (KBr, cm^{-1}): 1583.57 (C=N); ^{1}H NMR (400 MHz, DMSO-d6), δ ppm: 8.249 (s, 1H, HC=N), 7.877 (d, *J* = 7.6 Hz 2H, CH Ar), 7.765 (d, *J* = 7.6 Hz 2H, CH Ar), 7.353 (m, 7H, CH Ar), 7.197 (d, *J* = 8.0 Hz 2H, CH Ar), 6.771 (s, 1H, CH thiazole); ^{13}C NMR

(100 MHz, DMSO-d6), δ ppm: 168.1 (S-C), 150.4 (C=N-S), 148.8 (C=N), 145.20 (C, Ar) 137.2 (C thiazole), 133.7 (C,Ar), 133.4 (C,Ar), 132.8 (C,Ar), 129.7 (C, Ar), 129.5 (C, Ar), 128.5 (C, Ar), 127.3 (C, Ar), 126.3 (C, Ar), 125.1 (C, Ar), 125.3 (C, Ar), 124.5 (CF_3), 108.9 (C thiazole). Anal. Calcd for $C_{23}H_{15}ClF_3N_3S$: C, 60.33; H, 3.30; N, 9.18; S, 7. found: C, 58.80, H, 2.46; N, 8,99; S, 5.67. HRMS: 458.9000 [M+H] +

4.1.2.19 (E)-4-(4-fluorophenyl)-3-phenyl-2-{(E)-[4-(trifluoromethyl)benzylidene] hydrazono}-2,3-dihydrothiazole (3d)

Light Yellow crystals ; Yield 70%; m.p.(ºC) 205-208; Rf: 0,69 (hexane/ethyl acetate 7:3). IR (KBr, cm^{-1}): 1585.87 (C=N); ^{1}H NMR (400 MHz, DMSO-d6), δ ppm: 8.280 (s, 1H, HC=N), 7.880 (d, *J* = 8.1 Hz 2H, CH Ar), 7.764 (d, *J* = 8.7 Hz 2H, CH Ar), 7.358 (m, 4H, CH Ar), 7.225 (m, 4H, CH Ar), 7.086 (m, 1H,CH Ar), 6.787 (s, 1H, CH thiazole); ^{13}C NMR (100 MHz, DMSO-d6), δ ppm: 170.2 (C-F), 168.3 (S-C), 148.3 (C=N), 145.1 (C, Ar) 136.9 (C thiazole), 133.5 (C,Ar), 133.3 (C,Ar), 132.6 (C,Ar), 129.5 (C, Ar), 129.4 (C, Ar), 128.3 (C, Ar), 126.1 (C, Ar), 124.9 (C, Ar), 125.1 (C, Ar), 124.3 (CF3), 108.7 (C thiazole). Anal. Calcd for $C_{23}H_{15}F_4N_3S$: C, 62.58; H, 3.43; N, 17.21; S,7.26. found: C, 60.68, H, 2.79; N, 13.95; S, 6.12. HRMS: 441.4435 [M+H] +

4.2 Biological Activity

4.2.1 Ethical aspects for the alamar blue assay and hemolytic activity assay

The project was submitted to the Human Research Ethics Committee of the Federal University of Pernambuco, which is in accordance with Resolution 466/12 of the National Health Council and approved with CAAE opinion 62919816.2.0000.5208.

4.2.2 Cell culture

NCI-H292 (human lung carcinoma), HEp-2 (human larynx carcinoma) and HT-29 (human colon adenocarcinoma) were cultured in DMEM medium, while K-562 (human myeloblastic leukaemia) and HL-60 (human promyelocytic leukemia) were cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin, at 37 °C with 5% CO_2. The cell lines used in this work were obtained from Cell Bank of Rio de Janeiro

(BCRJ), Brazil. Culture medium was changed 2–3 days and subcultured when cell population density reached to 70–80% confluence.

4.2.3 Cytotoxicity assessment by MTT assay

The cytotoxicity of all compounds was tested using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) (Sigma Aldrich Co., St. Louis, MO/USA) against NCI-H292, HEp-2, HT-29, K562 e HL-60 lines cells [50]. Briefly, cells we added in 96-well plates (3 x 10^5 cells/mL for K562 and HL-60; and 1 x 10^5 cells/mL for NCI-H292, HT-29, and HEp-2) with appropriated medium and incubated for 24 h (37° C and 5% CO_2). Compounds were then added in different concentrations (0.098 to 25.0 µg/mL) and incubated for 72 h. Doxorubicin was used as a reference drug. Three hours before the end of the incubation time, 25 µL MTT (5 mg/mL in PBS) was added to each well. Culture medium was removed and 100 µL of DMSO were added. The amount of formazan was determined by measuring the absorbance at 560 nm. Concentration leading to 50% inhibition of viability (CI_{50}) was calculated by regression analysis using GraphPad Prism. Each sample was tested in triplicate in two independent experiments.

4.2.4 Alamar blue assay

To investigate the selectivity of synthetized compounds for tumor cells compared to normal proliferating cells, the Alamar blue assay was performed using PBMC (peripheral blood mononuclear cells) from healthy donors, after 72 h of drug exposure. All studies were performed in accordance with Research Guidelines (Law 196/96, National Council of Health). Heparinised blood (from healthy, non-smoker donors who had not taken any drug for at least 15 days prior to sampling) was collected, and the PBMC were isolated by a standard method of density-gradient centrifugation using Ficoll-Hypaque. PBMC were cultivated in RPMI-1640 medium supplemented with 20% foetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37 °C with 5% CO_2. Phytohaemagglutinin (2%) was added at the beginning of the culture period. Briefly, the PBMC were plated in 96-well plates (10^6 cells/mL). After 24 h, the compounds (0.048–25 µg/ mL), dissolved in DMSO, were added to each well and incubated for 72 h. Doxorubicin was used as a positive control. At 24 h before the end of the incubation, 10 µL of a stock solution (0.312 mg/mL) of Alamar blue (resazurin, Sigma-Aldrich Co., St. Louis, MO, USA)

was added to each well. The absorbance was measured using a plate reader, and the effect of the drug was quantified as the percentage of the control absorbance at 570 and 595 nm [51].

4.2.5 Haemolysis assay

The test was performed in 96-well plates using a 2% human erythrocyte suspension in 0.85% NaCl containing 10 mM $CaCl_2$ following the method described by Costa-Lotufo *et al.* [52] with modification. The diluted compounds were tested at concentrations ranging from 0.78 to 250 µg/mL. Triton X-100 (1%) was used as a positive control. After incubation at room temperature for 1 h followed by centrifugation, the supernatant was removed, and the liberated haemoglobin was measured spectrophotometrically at 540 nm.

4.2.6 Flow cytometry analysis

Cells of the K562 strain (3 x 10^5 cells/ml) were resuspended in RPMI-1640 medium and treated with **1d** compound for 48 h at 37°C with 5% CO_2. The cell viability assay was performed using the count detection kit (Guava technologies) and the cell cycle assay and DNA fragmentation using the guava cell cycle detection kit (Guava technologies) according to the manufacturer's instructions. The mitochondrial depolarization assay was performed using rhodamine. The experiment was performed on a Guava EasyCyteHT (Merck-Millipore) flow cytometer, acquiring 5,000 events. The data were analyzed in Guava-Soft™ software version 2.7. Two independent experiments were performed in triplicate.

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List of Captions

Figure 1. Inspiration process of structural planning. Anticancer agents I- flutamide, II-hydroxyflutamide, III- nilutamide, IV- 5-trifluoromethyl uracil [31], TP-07 [25], 4a, 7a and 10e [24].

Figure 2. Structural planning of the proposed series of compounds.

Scheme 1. Global synthesis of compounds **1-3d**. Reagents and conditions: (A) corresponding thiosemicarbazide, ethanol, HCl, reflux, 2-3 h; (B) corresponding α-haloketone, 2-propanol, rt, 1 h.

Table 1: Inhibition percentage ± SD of the derivatives of thiazoles and thiosemicarbazones *in vitro* by MTT assay, within 72 hours against tumor cells in the assay single dose of 25 µg/mL.
*NT - Not tested.s.

Table 2: IC_{50} values (µM) ± SD of compounds against different strains of human cancer.

Table 3. The cytotoxic potential of derivatives **1** and **1d** against K562 strain after 24, 48 and 72 hours of treatment.

Table 4. Hemolytic activity and cytotoxicity in peripheral blood mononuclear cells of the thiosemicarbazones and thiazoles derivatives.
*EC50: Effective concentration in which the test substance has cause haemolysis in 50% of erythrocytes. Results expressed in µg / mL and (µM).
**NT - Not tested.

Figure 3. Effects of compound **1d** and doxorubicin on the K562 cell line, determined by flow cytometry on mitochondrial depolarization, after 48 h of incubation. The negative control (A) was treated with the vehicle (DMSO) at 0.1%. Doxorubicin (B) 0.5 µM and **1d** (17 and 34 µM), C and D, respectively. Two independent experiments, each concentration in triplicate. *** $p < 0.0001$ in comparison to negative control (C).

Figure 4. Effects of compound **1d** and doxorubicin on the K562 cell line, determined by flow cytometry using cell cycle, after 48 h of incubation. The negative control (A) was treated with the vehicle (DMSO) at 0.1%. Doxorubicin (B) 0.2 µM and **1d** (17 and 34 µM), C and D, respectively. Two independent experiments, each concentration in triplicate. *** $p < 0.0001$ in comparison to negative control (C).

Figure 5. Effects of compound **1d** and doxorubicin on the K562 cell line, determined by flow cytometry on DNA fragmentation, after 48 h of incubation. The negative control (A) was treated with the vehicle (DMSO) at 0.1%. Doxorubicin (B) 0.5 µM and **1d** (17 and 34 µM) C and D, respectively. Two independent experiments, each concentration in triplicate. *** $p < 0.0001$ in comparison to negative control (C).

Table 5. Physicochemical properties of compounds. *PSA - Polar surface area.

Table 6: ADME properties of most active compounds.
*BBB - blood–brain barrier.
**GI - Gastrointestinal absorption.

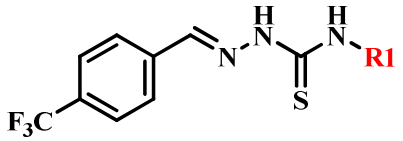
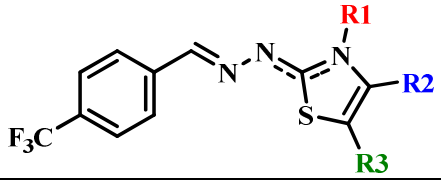
Code	R1	R2	R3	NCI-H292	HEp-2	HT-29	HL-60	K562
								
1	H	-	-	89,55 ± 3,93	91,75 ± 1,05	85,43 ± 1,47	95,35 ± 1,38	87,25 ± 1,46
2	Me	-	-	93,30 ± 1,04	88,13 ± 6,19	88,65 ± 0,64	94,64 ± 3,20	82,59 ± 0,38
3	Ph	-	-	58,38 ± 3,29	63,60 ± 0,83	74,67 ± 3,20	37,68 ± 5,06	71,16 ± 3,10
								
1a	H	Ph	H	49,79 ± 4,74	34,72 ± 3,34	NT	40,61 ± 1,33	NT
1b	H	Ph-4-NO_2	H	96,31 ± 2,80	89,99 ± 4,67	59,31 ± 3,05	80,25 ± 4,51	87,20 ± 1,53
1c	H	Ph-3-NO_2	H	75,10 ± 4,49	84,30 ± 9,46	79,43 ± 2,39	77,40 ± 5,71	75,46 ± 0,86
1d	H	Ph-2,4-diCl	H	96,53 ± 0,54	96,65 ± 0,43	79,47 ± 7,33	91,09 ± 5,22	88,89 ± 0,54
1e	H	Ph-4-MeO	H	68,52 ± 5,42	78,17 ± 5,84	76,22 ± 0,74	89,14 ± 1,49	61,50 ± 6,83
1f	H	Ph-4-Cl	H	67,70 ± 6,30	33,00 ± 4,49	40,14 ± 5,27	61,40 ± 5,71	50,50 ± 2,08
1g	H	Ph-4-Br	H	64,20 ± 4,20	17,10 ± 1,73	67,36 ± 6,67	46,70 ± 2,94	24,96 ± 1,90
1h	H	Ph-3,4-diCl	H	98,20 ± 7,26	83,50 ± 5,88	84,02 ± 2,80	96,40 ± 5,36	47,80 ± 2,68
1i	H	Ph	Me	60,90 ± 4,33	29,52 ± 2,32	46,20 ± 5,02	30,93 ± 1,39	25,04 ± 3,06
1j	H	2-Naph	H	35,69 ± 1,04	28,47 ± 2,69	45,80 ± 2,60	39,11 ± 1,66	44,89 ± 5,19
1k	H	Ph-4-Me	H	62,30 ± 6,05	59,93 ± 2,22	42,66 ± 3,35	45,20 ± 4,15	20,89 ± 0,26
1l	H	Ph-4-F	H	78,80 ± 3,63	62,60 ± 6,75	70,10 ± 2,42	58,10 ± 0,87	61,56 ± 2,37
2a	Me	Ph-4-MeO	H	68,30 ± 0,69	53,70 ± 6,74	62,46 ± 3,08	67,85 ± 3,36	50,42 ± 2,08
2b	Me	Ph-4-NO_2	H	44,07 ± 4,46	84,81 ± 2,86	75,10 ± 1,90	83,17 ± 0,96	59,79 ± 4,32
2c	Me	Ph-2,4-Cl	H	35,70 ± 3,29	33,48 ± 5,15	52,66 ± 2,94	43,47 ± 2,58	69,70 ± 0,69
3a	Ph	Ph-2,4-Cl	H	38,50 ± 1,04	*NT	*NT	14,40 ± 1,20	*NT
3b	Ph	Ph-4-MeO	H	66,30 ± 8,30	81,80 ± 2,42	58,50 ± 3,29	36,70 ± 1,21	59,20 ± 3,63
3c	Ph	Ph-4-Cl	H	87,80 ±11,59	58,70 ± 1,04	82,23 ± 1,49	28,41 ± 0,61	59,04 ± 2,52
3d	Ph	Ph-4-F	H	41,80 ± 1,90	21,97 ± 0,74	74,64 ± 9,03	26,21 ± 1,34	59,10 ± 4,73

Table 1: Inhibition percentage ± SD of the derivatives of thiazoles and thiosemicarbazones *in vitro* by MTT assay, within 72 hours against tumor cells in the assay single dose of 25 µg/mL.
*NT - Not tested.

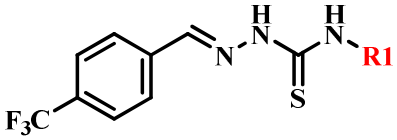
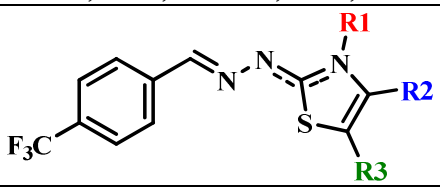
Code	R1	R2	R3	NCI-H292	HEp-2	HT-29	HL-60	K562
								
1	H	-	-	10,76 ± 1,38	6,27 ± 1,10	9,02 ± 1,31	12,74 ± 1,47	6,42 ± 1,08
2	Me	-	-	20,83 ± 4,60	12,58 ± 1,74	5,06 ± 0,65	17,22 ± 3,23	13,25 ± 2,25
								
1b	H	Ph-4'-NO_2	H	7,41 ± 1,37	27,33 ± 3,37	>63,56	11,51 ± 1,11	16,86 ± 3,23
1c	H	Ph-3'-NO_2	H	22,77 ± 2,77	34,24 ± 2,93	23,89 ± 3,22	29,29 ± 4,20	10,17 ± 1,50
1d	H	Ph-2',4'-diCl	H	10,41 ± 0,55	7,65 ± 0,83	11,15 ± 2,70	17,80 ± 2,80	5,48 ± 0,85
1e	H	Ph-4'-MeO	H	>66,17	11,31 ± 2,25	22,74 ± 8,31	3,67 ± 0,60	>66,17
1h	H	Ph-3',4'-diCl	H	6,57 ± 1,38	10,38 ± 2,15	12,21 ± 1,71	4,23 ± 0,81	>60,06
2b	CH_3	Ph-4'-NO_2	H	>61,37	26,39 ± 3,42	47,77 ± 4,71	11,83 ± 2,16	>61,37
Doxorrubicin	-	-	-	0,19 ± 0,04	1.21 ± 0,15	0,69 ± 0,15	0,19 ± 0,02	1,38 ± 0,15

Table 2: IC_{50} values (µM) ± SD of compounds against different strains of human cancer.

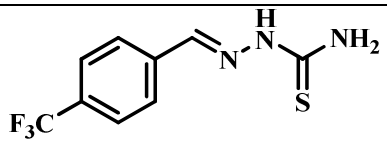
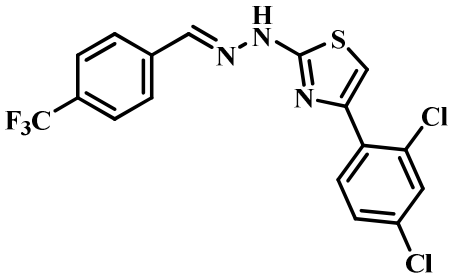
Code	Structure	IC_{50} (µM) ± SD		
		24 h	48 h	72 h
1		>101.12	33.98 ± 5,22	6.42 ± 1,08
1d		>60.06	17.78 ± 3,25	5.48 ± 0,85

Table 3. The cytotoxic potential of derivatives **1** and **1d** against K562 strain after 24, 48 and 72 hours of treatment.

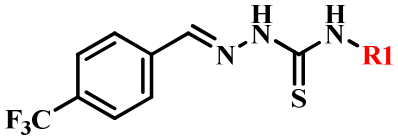
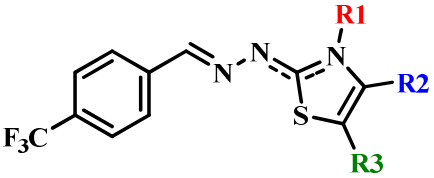
Code	R1	R2	R3	Hemolytic activity * (EC_{50})	PBMC (IC_{50})
					
1	H	-	-	> 250 µg/mL	80.17 µM
2	Me	-	-	> 250 µg/mL	93.31 µM
					
1b	H	Ph-4'-NO_2	H	> 250 µg/mL	53.16 µM
1c	H	Ph-3'-NO_2	H	> 250 µg/mL	150.52 µM
1d	H	Ph-2',4'-diCl	H	> 250 µg/mL	43.03 µM
1e	H	Ph-4'-MeO	H	> 250 µg/mL	130.40 µM
1h	H	Ph-3,4-diCl	H	> 250 µg/mL	65.56 µM
2b	CH_3	Ph-4'-NO_2	H	> 250 µg/mL	139.62 µM
Doxorrubicin	-	-	-	**NT	0.38 µM

Table 4. Hemolytic activity and cytotoxicity in peripheral blood mononuclear cells of the thiosemicarbazones and thiazoles derivatives.

*EC50: Effective concentration in which the test substance has cause haemolysis in 50% of erythrocytes. Results expressed in µg / mL and (µM).

**NT - Not tested.

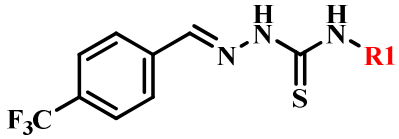
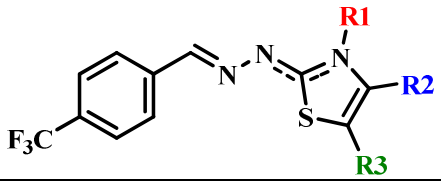
Code	R1	R2	R3	*BBB permeant	**GI absorption	Bioavailability Score
						
1	H	-	-	No	High	0.55
2	Me	-	-	No	High	0.55
						
1b	H	Ph-4-NO_2	H	No	Low	0.55
1c	H	Ph-3-NO_2	H	No	Low	0.55
1d	H	Ph-2,4-diCl	H	No	Low	0.55
1e	H	Ph-4-MeO	H	No	High	0.55
1h	H	Ph-3,4-diCl	H	No	Low	0.55
2b	Me	Ph-4-NO_2	H	No	Low	0.55
Doxorrubicin	-	-	-	No	Low	0.17

Table 5: ADME properties of most active compounds.
*BBB - blood–brain barrier.
**GI - Gastrointestinal absorption.

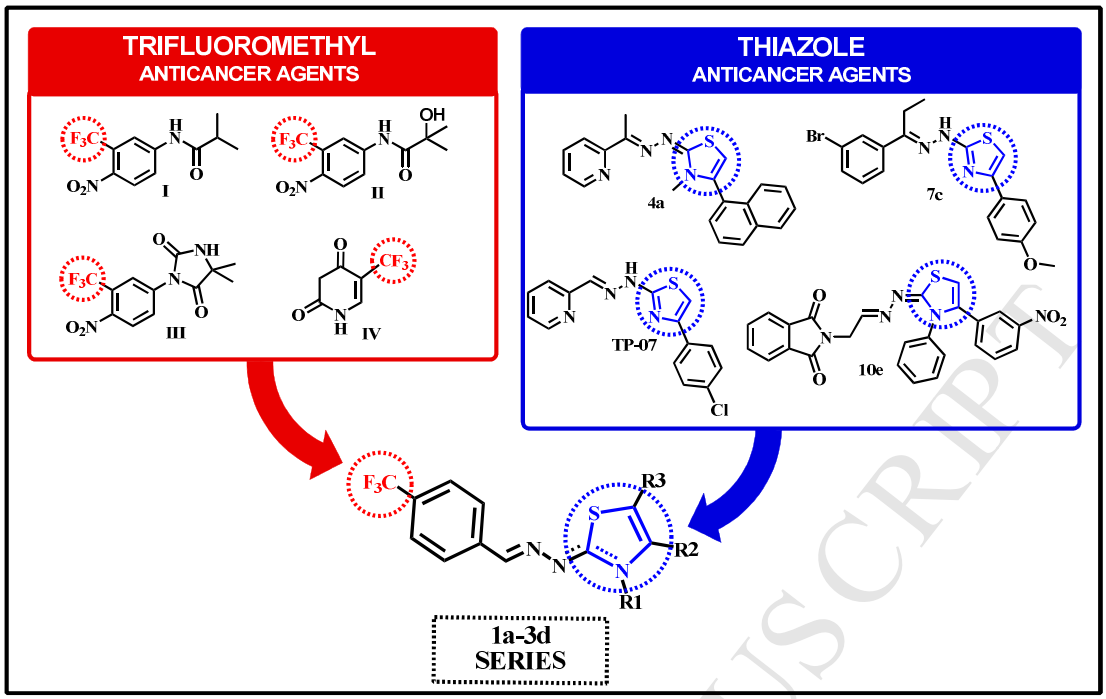


Figure 1. Inspiration process of structural planning. Anticancer agents I- flutamide, II- hydroxyflutamide, III- nilutamide, IV- 5-trifluoromethyl uracil [31], TP-07 [25], 4a, 7a and 10e [24].

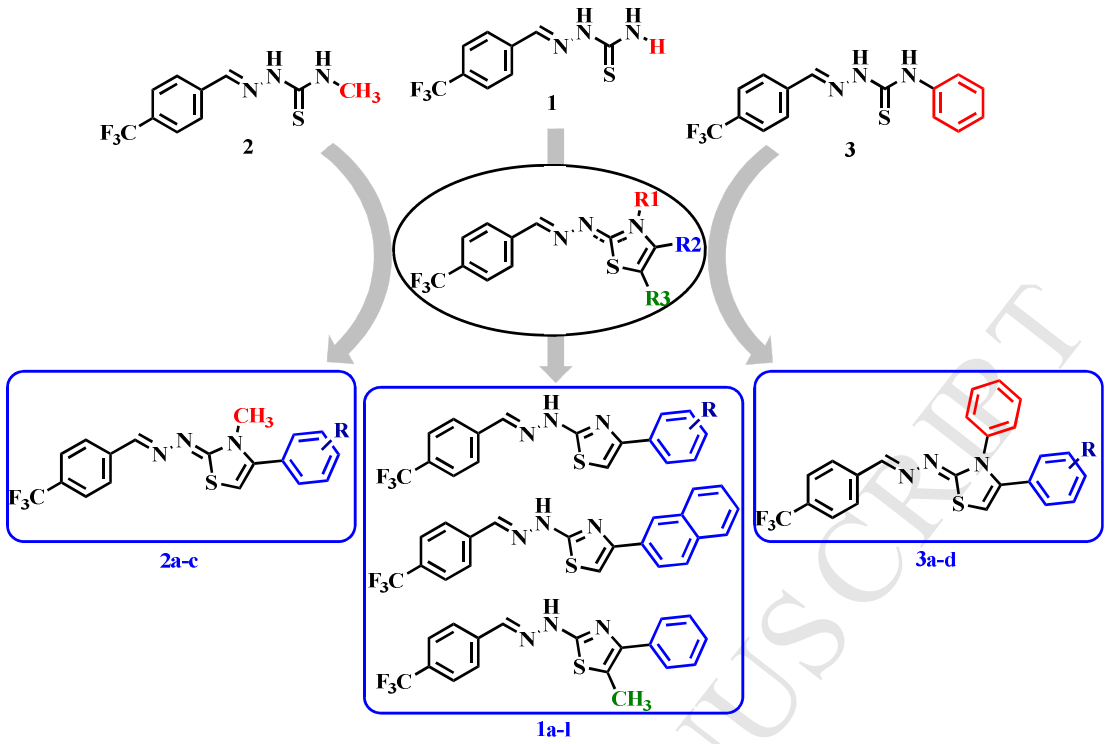
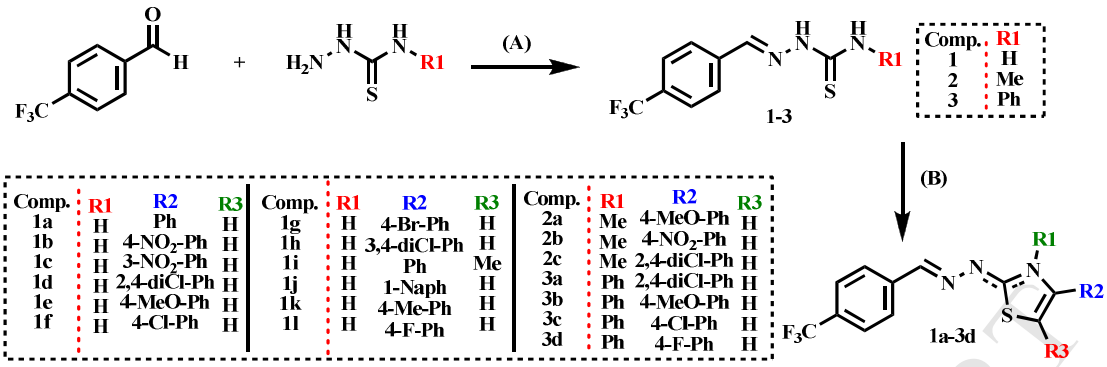
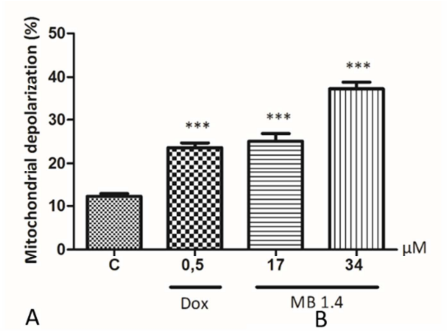


Figure 2. Structural planning of the proposed series of compounds.



Scheme 1. Global synthesis of compounds **1-3d**. Reagents and conditions: (A) corresponding thiosemicarbazide, ethanol, HCl, reflux, 2-3 h; (B) corresponding α-haloketone, 2-propanol, rt, 1 h.



Depolarization	% Cell
C	12,24 ± 0,61
Dox	23,60 ± 1,09
MB 1.4 – 17	25,05 ± 1,77
MB 1.4 – 34	37,12 ± 1,73

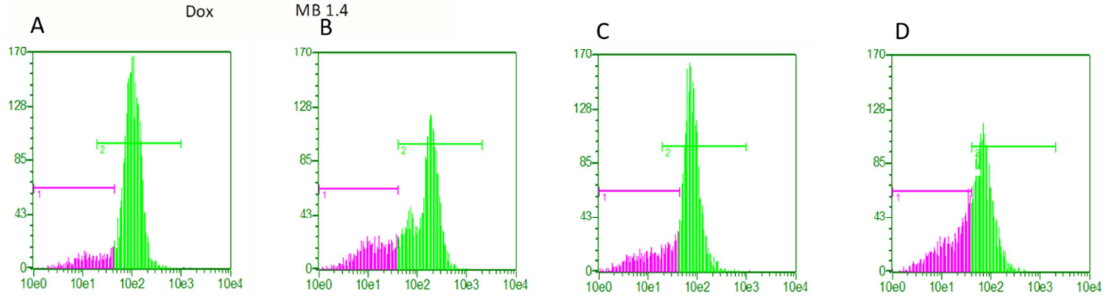
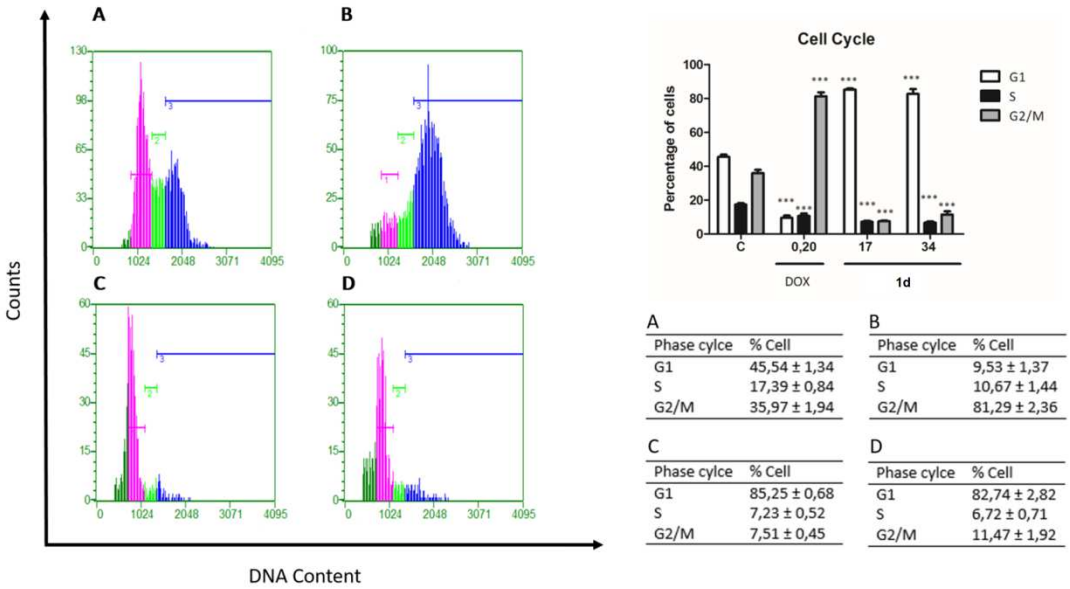


Figure 3. Effects of compound **1d** and doxorubicin on the K562 cell line, determined by flow cytometry on mitochondrial depolarization, after 48 h of incubation. The negative control (A) was treated with the vehicle (DMSO) at 0.1%. Doxorubicin (B) 0.5 µM and **1d** (17 and 34 µM), C and D, respectively. Two independent experiments, each concentration in triplicate. *** $p < 0.0001$ in comparison to negative control (C).



A

Phase cylce	% Cell
G1	45,54 ± 1,34
S	17,39 ± 0,84
G2/M	35,97 ± 1,94

B

Phase cylce	% Cell
G1	9,53 ± 1,37
S	10,67 ± 1,44
G2/M	81,29 ± 2,36

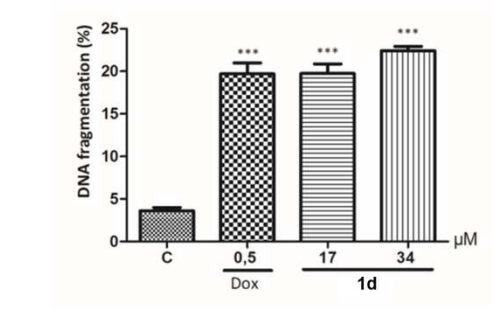
C

Phase cylce	% Cell
G1	85,25 ± 0,68
S	7,23 ± 0,52
G2/M	7,51 ± 0,45

D

Phase cylce	% Cell
G1	82,74 ± 2,82
S	6,72 ± 0,71
G2/M	11,47 ± 1,92

Figure 4. Effects of compound **1d** and doxorubicin on the K562 cell line, determined by flow cytometry using cell cycle, after 48 h of incubation. The negative control (A) was treated with the vehicle (DMSO) at 0.1%. Doxorubicin (B) 0.2 μM and **1d** (17 and 34 μM), C and D, respectively. Two independent experiments, each concentration in triplicate. *** $p < 0.0001$ in comparison to negative control (C).



Fragmentation	% Cell
C	3,62 ± 0,37
Dox	19,69 ± 1,30
1d - 17	19,74 ± 1,12
1d - 34	22,43 ± 0,51

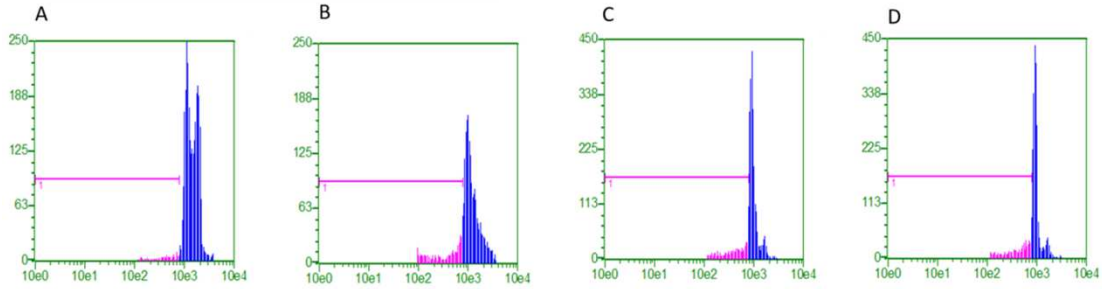


Figure 5. Effects of compound **1d** and doxorubicin on the K562 cell line, determined by flow cytometry on DNA fragmentation, after 48 h of incubation. The negative control (A) was treated with the vehicle (DMSO) at 0.1%. Doxorubicin (B) 0.5 µM and **1d** (17 and 34 µM) C and D, respectively. Two independent experiments, each concentration in triplicate. *** $p < 0.0001$ in comparison to negative control (C).

HIGHLIGHTS

The cytotoxic activity of 3 thiosemicarbazones and 19 new thiazoles was evaluated;

Eight compounds were shown to be promising in at least three tumor cell lines;

Compound **1d** induced mitochondrial depolarization;

Compound **1d** induced DNA fragmentation and arrest cell cycle in G1 phase;

Compound **1d** presented as a promising antitumor candidate.