Toxicology and Applied Pharmacology 329 (2017) 212–223

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

Anti-liver cancer activity in vitro and in vivo induced by 2-pyridyl 2,3-thiazole derivatives

Thiago David dos Santos Silva [a,b], Larissa Mendes Bomfim [c], Ana Carolina Borges da Cruz Rodrigues [c], Rosane Borges Dias [c], Caroline Brandi Schlaepfer Sales [d], Clarissa Araújo Gurgel Rocha [c], Milena Botelho Pereira Soares [c,e], Daniel Pereira Bezerra [c], Marcos Veríssimo de Oliveira Cardoso [f], Ana Cristina Lima Leite [a], Gardenia Carmen Gadelha Militão [b,*]

[a] Department of Pharmaceutical Sciences, Health Sciences Center, Federal University of Pernambuco, 50740-520 Recife, PE, Brazil
[b] Department of Physiology and Pharmacology, Federal University of Pernambuco, 50670-901 Recife, PE, Brazil
[c] Gonçalo Moniz Institute, Oswaldo Cruz Foundation (IGM-FIOCRUZ/BA), 40296-710 Salvador, Bahia, Brazil.
[d] Department of Biomorphology, Institute of Health Sciences, Federal University of Bahia, 40110-902 Salvador, Bahia, Brazil.
[e] Center of Biotechnology and Cell therapy, Hospital São Rafael, 41253-190 Salvador, Bahia, Brazil.
[f] Collegiate of Nutrition, University of Pernambuco, 56328-903 Petrolina, PE, Brazil

ARTICLE INFO

Article history:
Received 7 March 2017
Revised 15 May 2017
Accepted 6 June 2017
Available online 10 June 2017

Keywords:
2-Pyridyl 2,3-thiazoles
HepG2
Antitumor
Cytotoxicity
Liver Cancer
Toxicity

ABSTRACT

A total of 24 hybrid compounds containing pyridyl and 1,3-thiazole moieties were screened against HL-60 (leukemia), MCF-7 (breast adenocarcinoma), HepG2 (hepatocellular carcinoma), NCI-H292 (lung carcinoma) human tumor cell lines and non-tumor cells (PBMC, human peripheral blood mononuclear cells). Most of them were highly potent in at least one cell line tested ($IC_{50} \leq 3$ μM), being HL-60 the most sensitive and HepG2 the most resistant cell line. Among them, TAP-07 and TP-07 presented cytotoxic activity in all tumor cell lines, including HepG2 (IC_{50} 2.2 and 5.6 μM, respectively) without antiproliferative effects to normal cells (PBMC) ($IC_{50} > 30$ μM), making TAP-07 and TP-07, the compounds with the most favorable selectivity index. TAP-07 and TP-07 induced apoptosis in HepG2 cells and presented in vivo antitumor activity in hepatocellular xenograft cancer model in C.B-17 severe combined immunodeficient mice. Systemic toxicological verified by biochemical and histopathological techniques reveled no major signs of toxicity after treatment with TAP-07 and TP-07. Together the results indicated the anti-liver cancer activity of 2-pyridyl 2,3-thiazole derivatives.

1. Introduction

Cancer is the major public health problem worldwide. In the last two decades, improvements in diagnostics and therapeutics have been done, leading to 23% drop in the cancer death rate; however, cancer still is a leading cause of death worldwide. Therefore, basic and clinical research is necessary to reduce the mortality related to cancer (Siegel et al., 2016).

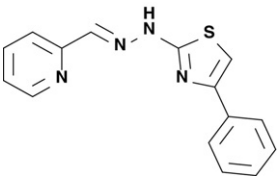
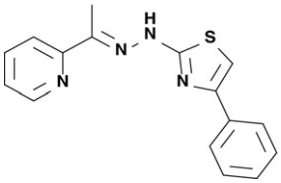
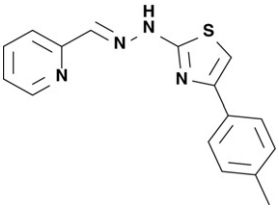
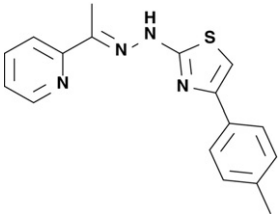
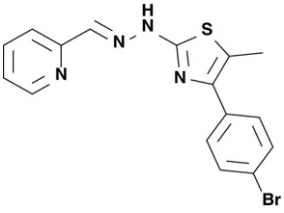
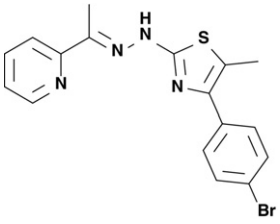
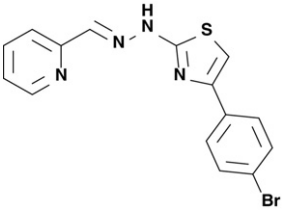
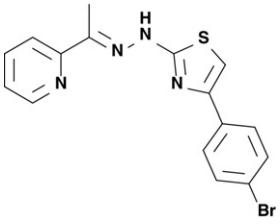
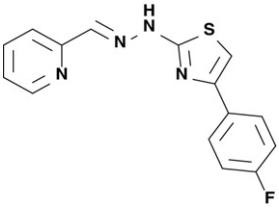
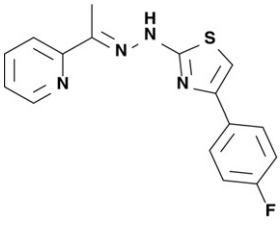
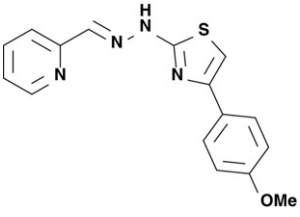
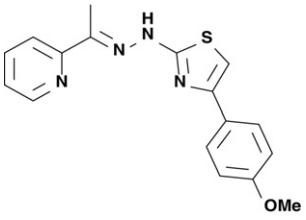
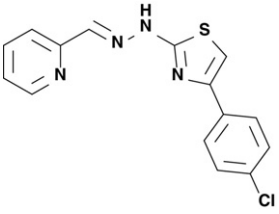
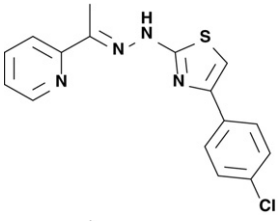
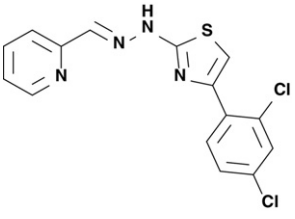
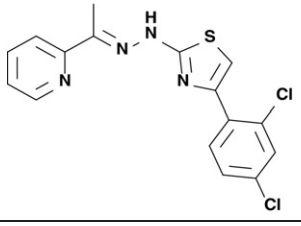
Thiosemicarbazones present many biological activities, such as antibacterial, antifungal (Beraldo and Gambinob, 2004), trypanocidal (da S Maia et al., 2009), larvicidal (da Silva et al., 2015), immunomodulatory and antitumor (dos Santos et al., 2016). Since the development of triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone, a ribonucleotide reductase inhibitor), much attention has been given to pyridine thiosemicarbazones (Niu et al., 1995). Pyridine, a basic heterocyclic compound with molecular formula C_5H_5N, and its derivatives are compounds with various applications in medicinal area (Altaf et al., 2015). As anticancer drugs, di-2-pyridylketone thiosemicarbazone, 2-benzoylpyridine thiosemicarbazone and 2-acetylpyridine thiosemicarbazone were developed as cytotoxic compounds targeting metal ions, which are essential nutrients for many cellular processes (Jansson et al., 2015).

The derivatization of 2-pyridyl thiosemicarbazone group into thiazole moiety generated novel templates with increased trypanocidal activity, (Cardoso et al., 2014). The thiazole ring acts as pharmacophore with versatile scaffold for the synthesis of new derivatives. In the cancer research, thiazole derivatives can act though the inhibition of kinases, inhibition of matrix metalloproteinases (MMPs) and inhibition of anti-apoptotic BCL2 family proteins (Morigi et al., 2015). In addition, novel thiazole analogues presented antimetastatic properties (Morigi et al., 2015). The strategy to connect pyridyl and thiazole ring into a new series of tricyclic compounds was used to develop molecules targeting the pro domain of the inactive metalloproteinases zymogens, proMMPs.

* Corresponding author at: Department of Physiology and Pharmacology, Federal University of Pernambuco, Rua Prof. Moraes Rego, Cidade Universitária, CEP 50.670-901 Recife, PE, Brazil.
E-mail addresses: gcgadelha@yahoo.com.br, gardenia.militao@ufpe.br (G.C.G. Militão).

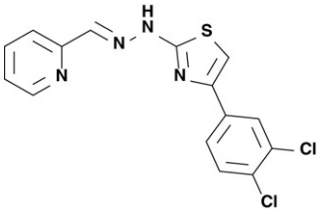
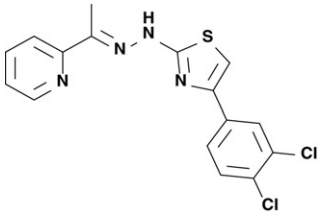
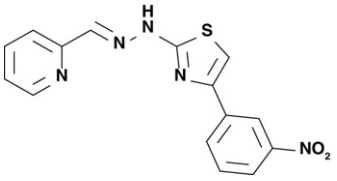
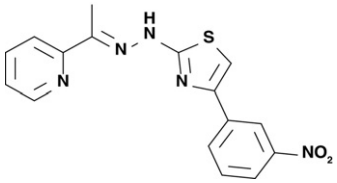
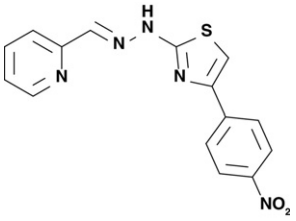
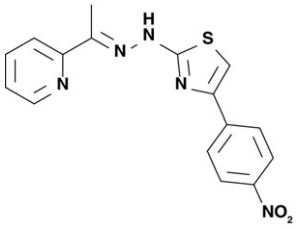
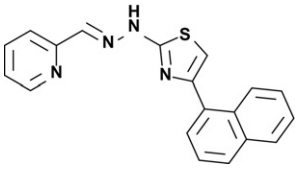
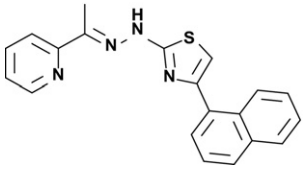
http://dx.doi.org/10.1016/j.taap.2017.06.003
0041-008X/

Table 1
Structures of 2-pyridyl 2,3-thiazole derivatives.

Cpd	Structure	Cpd	Structure
TP-01		TAP-01	
TP-02		TAP-02	
TP-03		TAP-03	
TP-04		TAP-04	
TP-05		TAP-05	
TP-06		TAP-06	
TP-07		TAP-07	
TP-08		TAP-08	

(continued on next page)

Table 1 (*continued*)

Cpd	Structure	Cpd	Structure
TP-09		TAP-09	
TP-10		TAP-10	
TP-11		TAP-11	
TP-12		TAP-12	

For malignant cells, MMPS can regulate the tumor microenvironment, cleavage substrate at the connective tissue and help cancer cells to invade surrounding barriers and metastasize (Egeblad and Werb, 2002). Thus, pyridyl-thiazolyl compounds were developed to inhibiting the proteolytic activation of pro-matrix MMP9 and pro-MMP1 (Zhang et al., 2011). Hybrid compounds containing pyridyl and 1,3-thiazole moieties were described as cytotoxic to cancer cells, immunomodulatory and anti-inflammatory compounds (dos Santos et al., 2016; Miwatashi et al., 2005). Based on the pyridyl-thiazolyl compounds potential as anticancer agents, we investigated the anticancer activity of 2-pyridyl 2,3-thiazole derivatives, both in vitro and in vivo.

2. Material and methods

2.1. Compounds

Compounds 2-(2-(pyridin-2-ylmethylene)hydrazinyl)-4-phenyl-1,3-thiazole (TP-01), 2-(2-(pyridin-2-ylmethylene)hydrazinyl)-4-(4-tolyl)-1,3-thiazole (TP-02), 2-(2-(pyridin-2-ylmethylene)hydrazinyl)-4-(4-bromophenyl)-1,3-thiazole (TP-03), 5-methyl-2-(2-(pyridin-2-ylmethylene)hydrazinyl)-4-(4-bromophenyl)-1,3-thiazole (TP-04), 2-(2-(pyridin-2-ylmethylene)hydrazinyl)-4-(4-fluorophenyl)-1,3-thiazole (TP-05), 2-(2-(pyridin-2-ylmethylene)hydrazinyl)-4-(4-methoxyphenyl)-1,3-thiazole (TP-06), 2-(2-(pyridin-2-ylmethylene)hydrazinyl)-4-(4-chlorophenyl)-1,3-thiazole (TP-07), 2-(2-(pyridin-2-ylmethylene)hydrazinyl)-4-(2,4-dichlorophenyl)-1,3-thiazole (TP-08), 2-(2-(pyridin-2-ylmethylene)hydrazinyl)-4-(3,4-dichlorophenyl)-1,3-thiazole (TP-09), 2-(2-(pyridin-2-ylmethylene)hydrazinyl)-4-(3-nitrophenyl)-1,3-thiazole (TP-10), 2-(2-(pyridin-2-ylmethylene)hydrazinyl)-4-(4-nitrophenyl)-1,3-thiazole (TP-11), 2-(2-(pyridin-2-ylmethylene)hydrazinyl)-4-(naphthalen-1-yl)-1,3-thiazole (TP-12), (2-(1-(pyridin-2-yl)ethylene)hydrazinyl)-4-phenyl-1,3-thiazole (TAP-01), (2-(1-(pyridin-2-yl)ethylene)hydrazinyl)-4-(4-tolyl)-1,3-thiazole (TAP-02), (2-(1-(pyridin-2-yl)ethylene)hydrazinyl)-4-(4-bromophenyl)-1,3-thiazole (TAP-03), 5-methyl-(2-(1-(pyridin-2-yl)ethylene)hydrazinyl)-4-(4-bromophenyl)-1,3-thiazole (TAP-04), (2-(1-(pyridin-2-yl)ethylene)hydrazinyl)-4-(4-fluorophenyl)-1,3-thiazole (TAP-05), (2-(1-(pyridin-2-yl)ethylene)hydrazinyl)-4-(4-methoxyphenyl)-1,3-thiazole (TAP-06), (2-(1-(pyridin-2-yl)ethylene)hydrazinyl)-4-(4-chlorophenyl)-1,3-thiazole (TAP-07), (2-(1-(pyridin-2-yl)ethylene)hydrazinyl)-4-(2,4-dichlorophenyl)-1,3-thiazole (TAP-08), (2-(1-(pyridin-2-yl)ethylene)hydrazinyl)-4-(3,4-dichlorophenyl)-1,3-thiazole (TAP-09), (2-(1-(pyridin-2-yl)ethylene)hydrazinyl)-4-(3-nitrophenyl)-1,3-thiazole (TAP-10), (2-(1-(pyridin-2-yl)ethylene)hydrazinyl)-4-(4-nitrophenyl)-1,3-thiazole (TAP-11) and (2-(1-(pyridin-2-yl)ethylene)hydrazinyl)-4-(naphthalen-1-yl)-1,3-thiazole (TAP-12) were prepared by the methods previously reported by Cardoso et al. (2014) (Table 1). All compounds were chemically characterized by nuclear magnetic resonance (NMR), infrared, and mass spectra and by elemental analysis and presented purity of 95%.

2.2. In vitro assays

2.2.1. Cytotoxic assay. Human tumor cell lines HL-60 (promyelocytic leukemia), MCF-7 (breast adenocarcinoma), NCI-H292 (lung carcinoma) and HepG2 (hepatocellular carcinoma), obtained from Rio de Janeiro Cell Bank (Rio de Janeiro, Brazil), were used. In addition, human peripheral blood mononuclear cells (PBMCs) obtained from healthy volunteers were also used. The PBMCs were isolated according to the standard method of density-gradient centrifugation over Ficoll-Histopaque. The protocol (number 1020660) was approved by the Ethics Committee of the Federal University of Pernambuco. The cells were maintained in RPMI 1640 medium or DMEN supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin,

100 μg/mL streptomycin (Gigco™ Life Technologies, USA) at 37 °C with 5% CO_2. To assess the cytotoxicity of the compounds, the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT, Sigma-Aldrich-USA) reduction assay was used after 72 h incubation. For all experiments, cells were plated in 96-well plates (10^5 cells/mL for adherent cells or 3×10^5 cells/mL for the leukemia) (Mosmann, 1983). After 24 h, the compounds, diluted in complete medium with 0.5% dimethylsulfoxide (DMSO, Vetec, Brazil), in concentrations ranging from 0.3 to 25 μg/mL were added to each well. Negative control received the same amount of DMSO. Doxorubicin (Sigma-Aldrich, USA) (0.01–5 μg/mL) was used as the positive control. After 69 h of treatment, 20 μL of MTT (5 mg/mL, Sigma Aldrich Co., St. Louis, MO, USA) was added. In the end of the incubation, the MTT formazan product was dissolved in 100 μL of DMSO and the absorbance was measured at 595 nm in a plate spectrophotometer (Varioskan Flash; Thermo Scientific, Finland).

2.2.2. Hemolytic assay. The hemolytic assay was performed with human erythrocytes obtained from peripheral blood of healthy volunteers (Fortes et al., 2016). The protocol (Number 1020660) was approved by the Ethics Committee of the Federal University of Pernambuco. For the experiment, 100 μL of a 2% erythrocytes suspension in 0.85% NaCl (Alphatec- Brazil) containing 10 mM $CaCl_2$ (Vetec-Brazil) was added to each well. A solution of triton X-100 (Vetec, Brazil) (1% w/v) was used as the positive control. Saline solution (0.85% NaCl containing 10 mM $CaCl_2$) was used as the negative control and the samples were tested in a concentration of 500 μM. After incubation at room temperature for 1 h, the supernatant was removed and the liberated hemoglobin was measured at 540 nm in a plate spectrophotometer (Varioskan Flash; Thermo Scientific, Finland).

2.2.3. Cell viability by trypan blue exclusion assay. Trypan blue exclusion assay was used to determinate the direct effect of the drugs in the cell viability. The number of viable cells and non-viable (take up trypan blue) were counted. Briefly, 90 μL was removed from the cell suspension and 10 μL of trypan blue (0.4%) was added. Cell counting was performed using a Leica DM500 microscope (Leica Microsystems, Switzerland) with a hemocytometer filled with an aliquot of the homogenized cell suspension.

2.2.4. Cell morphology. To evaluate alterations in cell morphology, slides were prepared using cytospin. The cells were fixed with 96% ethanol for 5 min and stained with May-Grunwald-Giemsa. Cellular changes were analyzed by light microscope and photographed with NIS-Elements imaging software (Nikon, model Eclipse NI-U, Japan).

2.2.5. Internucleosomal DNA fragmentation and cell cycle distribution. Cells were harvested in a permeabilization solution containing 0.1% triton X-100 (Sigma Chemical Co. St Louis, MO, USA), 2 μg/mL propidium iodide (Sigma Chemical Co. St Louis, MO, USA), 0.1% sodium citrate and 100 μg/mL RNAse (Sigma Chemical Co. St Louis, MO, USA). Cell fluorescence was determined by flow cytometry in a BD LSRFortessa cytometer (BD Biosciences, San Jose, CA, USA) using the BD FACSDiva Software (BD Biosciences, San Jose, CA, USA) and Flowjo Software 10 (Flowjo LCC, Ashland, OR, EUA). Ten thousand events were evaluated per experiment and cellular debris were omitted from the analysis.

2.2.6. Annexin V assay. Phosphatidylserine externalization was analyzed by flow cytometry. A FITC annexin V apoptosis detection kit (BD Biosciences, San Jose, CA, USA) was used to determine cell viability (viable, early apoptotic, late apoptotic and necrotic cells). Cells were washed twice with saline and then resuspended in 100 μL of binding buffer with 5 μL of propidum iodide and 5 μL of FITC annexin V. The cells were gently mixed on a vortex and incubated for 15 min at room temperature in the dark. Afterwards, 400 μL of binding buffer was added to each tube and the cells were analyzed by flow cytometry on a BD LSRFortessa cytometer using the BD FACSDiva Software and Flowjo Software 10. Ten thousand events were evaluated per experiment and cellular debris was omitted from the analysis.

2.3. In vivo assays

2.3.1. Animals. A total of 66 C·B-17 severe combined immunodeficient (SCID) mice (females, 25–30 g) was obtained and maintained at the animal facilities from Gonçalo Moniz Institute-FIOCRUZ (Salvador, Bahia, Brazil). Animals were housed in cages with free access to food and water. All animals were kept under a 12:12 h light-dark cycle (lights on at 6:00 a.m.). Animals were treated according to the ethical principles for animal experimentation of SBCAL (Brazilian association of laboratory animal science), Brazil. The local Animal Ethics Committee approved the experimental protocol (number 06/2015).

2.3.2. Human tumor xenograft model. HepG2 cells (10^7 cells per 500 μL in 0.9% NaCl) were implanted subcutaneously into the left front hand of the mice. Mice were randomly divided into four groups at the beginning of the experiment: group 1: animals that received injection of the vehicle, 5% DMSO solution (negative control, n = 15); group 2: animals that received injection of 5-fluorouracil (10 mg/Kg/day, positive control, n = 9, purity > 99%, Sigma Chemical Co. St Louis, MO, USA); group 3: animals that received injection of TAP-07 (40 mg/kg/day, n = 15); group 4: animals that received injection of TP-07 (40 mg/kg/day, n = 15); The treatments were initiated one day after tumor injection. Animals were treated intraperitoneally (200 μL per animal) once daily for 21 consecutive days. On the 22th day, animals were anesthetized, with ketamine/xylazine, and peripheral blood samples were collected from brachial artery. Animal were euthanized by anesthetic overdose and tumors were excised and weighed. The drug effects were expressed as the percent inhibition of control.

2.3.3. Systematic toxicological evolution. To perform the systematic toxicological effects, mice were weighed at the beginning and end of the experiment. Animals were observed for signs of abnormalities throughout the study. Hematological analyses were performed using light microscopy. Biochemical analyses of serum samples were performed using a Vet-16 rotor and quantified using an Analyst benchtop clinical chemistry system (Hemagen Diagnostics, Inc., Columbia, MD, EUA). Livers, kidneys, lungs and hearts were removed, weighed and examined for any signs of gross lesions, colour changes and/or hemorrhage. After gross macroscopic examination, tumors, livers, kidneys, lungs and hearts were fixed in 4% formalin buffer and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin chromogens, and a pathologist performed analyses under light microscopy.

2.4. Statistical analysis

Data are presented as mean ± S.E.M., inhibitory concentration of 50% (IC_{50}) or effective concentration of 50% (EC_{50}) values and their 95% confidence intervals obtained by nonlinear regression from at the least two independent experiments performed in duplicate or triplicate. Differences among experimental groups were compared by one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test ($p < 0.05$). All analyses were carried out using the GRAPHPAD PRISM program (Intuitive Software for Science).

3. Results

3.1. In vitro assays

3.1.1. 2-pyridyl 2,3-thiazole compounds displayed potent cytotoxicity to different tumor cell lines. Twenty-four compounds were evaluated for cytotoxicity toward four cancer cell lines (HL-60, MCF-7, NCI-H292 and HepG2). The compounds were classified into two groups, according to the chemical structure what they are derived: TP compounds are 2-pyridyl 2,3-thiazole compounds and TAP group are 2-pyridyl 2,3-thiazoles with a methyl at C6. Most of TAP and TP compounds presented potent cytotoxicity in at least one cell line tested (Table 2). The most sensitive cell line was HL-60 (promyelocytic leukemia) and the most resistant cell line was HepG2 (hepatocellular carcinoma). Compounds with $IC_{50} \leq 3$ μM were judged as highly potent, whereas compounds bearing IC_{50} values in the range of 3–6 μM were regarded as potent compounds. On the other hand, compounds with IC_{50} between 6 μM and 12 μM were considered as moderately potent, while molecules displaying IC_{50} values in the range of 12–24 μM were regarded as bearing low potency. Finally, the compounds exhibiting $IC_{50} > 24$ μM were deemed not cytotoxic (Fortes et al., 2016). In TAP group, TAP-07 presented cytotoxic activity at all cell lines tested with IC_{50} ranging from 0.98 to 3.5 μM. Even at HepG2 cell line, which was in general more resistant, TAP-07 was highly potent with IC_{50} of 2.2 μM. In regard to the toxicity in non-tumor PBMC cells, most of the TAP compounds were cytotoxic to PBMC, with exception to TAP-07 that was not cytotoxic at concentrations tested ($IC_{50} > 30$ μM), making TAP-07 the compound with the most favorable selectivity index (SI). Among compounds belonging to

Table 2
Cytotoxic activity of TAP and TP compounds.

Compound	IC_{50} in μM (Confidence interval)				
	HL-60	MCF-7	NCI-H292	HepG2	PBMC
TAP-01	0.91 (0.71–1.2)	1.01 (0.7–2.2)	1.8 (1.3–2.4)	>50	<3.5
TAP-02	0.64 (0.42–1.0)	2.1 (0.77–5.1)	2.4 (1.9–3.1)	>50	6.9 (5.5–13.6)
TAP-03	0.75 (0.56–0.95)	1.1 (0.7–1.7)	1.9 (1.5–2.3)	>50	13.8 (7.4–26.0)
TAP-04	0.51 (0.43–0.64)	1.61 (0.75–3.22)	1.4 (1.0–1.8)	>50	3.7 (2.3–6.6)
TAP-05	0.70 (0.57–0.89)	1.5 (1.1–1.8)	2.6 (1.5–4.7)	>50	13.1 (7.3–22.9)
TAP-06	0.8 (0.58–1.07)	1.3 (1.1–1.6)	3.0 (1.5–5.8)	>50	<3.5
TAP-07	0.98 (0.82–1.15)	3.5 (1.4–9.1)	3.1 (2.7–4.9)	2.2 (1.5–3.3)	>30
TAP-08	1.62 (1.40–1.90)	1.5 (0.44–5.2)	3.6 (1.8–4.3)	>50	<3.5
TAP-09	0.66 (0.44–0.93)	3.9 (1.8–8.5)	2.7 (2.4–3.1)	>50	<3.5
TAP-10	0.58 (0.41–0.85)	1.2 (0.91–1.5)	3.0 (1.9–4.7)	>50	<3.5
TAP-11	0.85 (0.67–0.94)	1.01 (0.85–1.15)	1.7 (1.4–2.5)	>50	4.0 (2.3–6.7)
TAP-12	1.4 (1.10–1.80)	2.7 (1.4–5.4)	2.7 (2.2–3.4)	>50	6.6 (4.6–9.3)
TP-01	1.7 (1.4–2.4)	11.0 (5.7–21.5)	10.7 (6.4–17.8)	>50	6.6 (13.9–33.3)
TP-02	1.3 (1.1–1.5)	3.8 (3.0–4.9)	7.5 (5.1–11.0)	6.1 (4.3–8.6)	<3.5
TP-03	27.3 (20.9–35.5)	4.4 (1.6–11.2)	2.2 (1.1–3.1)	>50	9.0 (7.5–8.6)
TP-04	1.3 (1.0–1.7)	2.5 (2.2–3.2)	6.4 (4.1–10.0)	>50	<3.5
TP-05	1.9 (1.6–2.2)	4.6 (3.7–5.7)	14.6 (8.3–25.4)	>50	7.9 (3.2–18.8)
TP-06	8.7 (5.0–14.8)	5.7 (2.6–12.4)	2.8 (1.5–5.0)	>50	<3.5
TP-07	1.0 (0.85–1.2)	4.7 (3.2–6.8)	3.2 (2.7–3.8)	5.8 (4.2–7.9)	>30
TP-08	0.57 (0.22–0.97)	2.5 (1.9–3.3)	0.94 (0.45–2.0)	>50	12.3 (7.7–19.0)
TP-09	1.2 (0.17–9.1)	2.9 (1.4–5.7)	2.4 (1.8–2.8)	>50	13.0 (6.6–25.5)
TP-10	0.98 (0.67–1.4)	4.3 (2.2–7.9)	3.3 (3.0–3.6)	>50	>30
TP-11	2.7 (2.2–3.4)	>50	>50	>50	>30
TP-12	3.1 (2.4–4.0)	7.2 (5.7–9.0)	>50	>50	19.3 (12.7–29.0)
DOX	0.02 (0.02–0.04)	0.37 (0.18–0.92)	0.55 (0.37–0.92)	0.6 (0.51–0.81)	1.4 (0.9–2.6)

Data are presented as IC_{50} values in μM and their respective 95% confidence interval obtained by nonlinear regression from at the least two independent experiments performed in duplicate or triplicate, measured by MTT reduction assay after 72 h incubation. Tumor cells: HL-60 (human promyelocytic leukemia); MCF-7 (human breast adenocarcinoma); NCI-H292 (human lung carcinoma); and HepG2 (human hepatocellular carcinoma). Non-tumor cells: PBMC (human peripheral blood mononuclear cells). Doxorubicin (DOX) was used as the positive control.

TP group, only TP-02 and TP-07 were cytotoxic to all cell lines tested. Conversely, only compound TP-02 was cytotoxic to PBMC (IC_{50} of 5.8 μM) when compared to TP-07, which was not cytotoxic at concentrations tested (IC_{50} > 30 μM). Compounds that cause non-specific cell death targeting the cell plasmatic membrane are very toxic. A positive hemolytic assay excludes the compound from being used as therapeutic drug. None of the compounds tested were toxic to human erythrocytes (EC_{50} > 250 μM). The cytotoxicity of TAP-07 and TP-07 stood out in relation to the other series of compounds synthetized, in particular due to their cytotoxic potential to HL-60 and HepG2 cells and the lack of toxicity to PBMC (Table 2). Thus, TAP-07 and TP-07 were selected for further experiments.

3.1.2. 2-Pyridyl 2,3-thiazole compounds induce apoptotic cell death in HepG2 cell line. HepG2 is an adherent liver tumor cell line which forms solid tumor when implanted to the animal. In order to check if the compounds are capable to act on suspension cancer cells, a leukemic cell line (HL60) was also treated with TAP-07 and TP-07. HL-60 and HepG2 cells were treated with TAP-07 and TP-07 for 24 and 48 h and the cell viability was evaluated using trypan blue dye exclusion method. Both compounds reduced the cell viability (Fig. 1). In HepG2 cells, TAP-07 caused significant ($p < 0.05$) cell growth inhibition at 7.5 (69%) and 15 μM (82%) after 24 h, while viable cell number reduced by 42 and 55% after treatment with TP-07 at 16 and 32 μM, respectively. After 48 h, TAP-07 caused significant ($p < 0.05$) cell growth inhibition at 8 (85%) and 16 μM (93%) and TP-07, at 16 and 32 μM, reduced by 68 and 80%, respectively. In HL-60 cells, viable cells number treated for 24 h with TAP-07 0.8 and 1.6 μM were reduced by 79 and 89%, respectively. The HL60 cell number was also reduced after treatment with TP-07 6 (46% of inhibition) and 12 μM (53.7% of inhibition). After 48 h incubation, TAP-07 caused significant ($p < 0.05$) cell growth inhibition at 0.8 (87%) and 1.6 μM (91%) and TP-07, at 6 and 12 μM, reduced by 57 and 63%, respectively. Doxorubicin, at 2 μM, reduced HepG2 cell number by 59 and 67% and HL-60 cells number by 72.2 and 79% after 24 and 48 h, respectively.

Morphological analysis performed after May-Grunwald-Giemsa staining showed that both HepG2 and HL-60 cell lines treated with TAP-07 and TP-07 for 24 and 48 h presented fragmentation of the nuclei that became more evident at higher concentrations. A reduction on cell volume was frequently verified in cells with altered nuclei (Fig. 2). In the negative controls, mitotic cells were found indicating the proliferative status of the cell lines, as well as round shaped interphase cell nucleus.

Analyses of the DNA distribution by flow cytometry were also included in the study. Significant ($p < 0.05$) internucleosomal DNA fragmentation of 17.2 ± 2.4%, 25.5 ± 4.0% and 17.8 ± 4.4% was found in HepG2 cells after 24 h of treatment with TAP-07 at 8 and 16 μM and TP-07 at 32 μM, respectively, compared to the control (5.9 ± 2.0%) (Fig. 3). The treatment of HepG2 cells with TAP-07 (7.5 and 15 μM) and TP-07 (32 μM) reduced the number of cells at G_0/G_1 ($p < 0.05$). The S phase and G_2/M phases of the cell cycle were not affected by TAP-07 nor TP-07 treatment. After 48 h of treatment an increase ($p < 0.05$) of the internucleosomal DNA fragmentation in HepG2 cells was also observed after treatment with TAP-07 at 8 and 16 μM (22 and 29% of sub-G_1 population, respectively, compared to 3.8% of the negative control). TAP-07 significantly ($p < 0.05$) reduced the number of HepG2 cells at G_2/M after 48 h of treatment. The G_2/M population of the negative control was 18.2 ± 2.4%, while for the cells treated with TAP-07 at 8 and 16 μM, the percentage of cells at G_2/M was 6.0 ± 0.6 and 4.9 ± 1.1, respectively. Quantitative analyses by flow cytometry reveled an increase on fragmented DNA content of HL-60 cells treated with TAP-07 0.8 and 1.6 μM and TP-07 12 μM (sub-G_1 population was 6.9 ± 2.0% at negative control and increased to 17.5 ± 7.0%, 20.8 ± 6.0% and 12.4 ± 2.0 after 24 h of treatment with TAP-07 0.8, 1.6 μM and TP-07 12 μM respectively. When HL60 cells were treated with TAP-07 and TP-07 for 48 h, the effect of TAP-07 and TP-07 on fragmented DNA induction was more potent.

The externalization of phosphatidylserine was evaluated by annexin V/propidium iodite staining. The percentage of HepG2 annexin V positive cells (apoptosis) was significantly higher after 24 h of treatment with TAP-07 and TP-07 at 16 μM (7.9 ± 0.6%) and 32 μM (9.0 ± 3.3%)

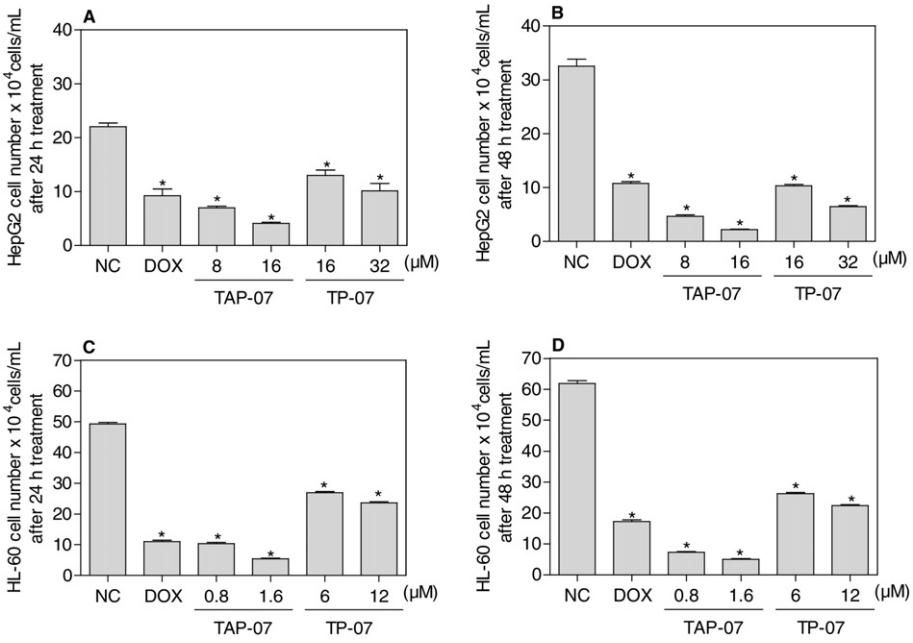


Fig. 1. Cell viability determined by trypan blue staining of HepG2 (A and B) and HL-60 (C and D) cells treated with TAP-07 and TP-07 after 24 (A and C) and 48 (B and D) h incubation. The negative control (NC) was treated with the vehicle (0.1% DMSO) used for diluting the substance tested. Doxorubicin (DOX, 2 μM) was used as the positive control. Data are presented as the mean ± S.E.M. of at the least three independent experiments performed in duplicate. *$p < 0.05$ compared with the negative control by ANOVA followed by Dunnett's Multiple Comparison Test.

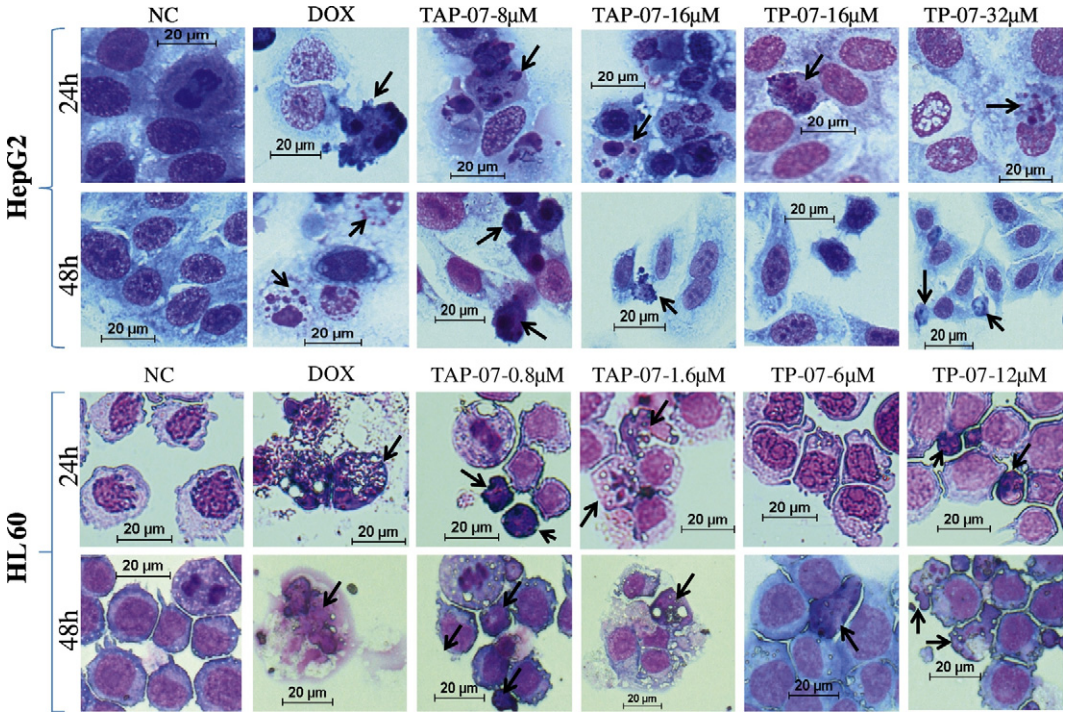


Fig. 2. Microscopic analysis of HepG2 and HL-60 cells stained with May-Grunwald-Giemsa after 24 and 48 h incubation. The negative control (NC) was treated with the vehicle (0.1% DMSO) used for diluting the substance tested. Doxorubicin (DOX, 2 μM) was used as the positive control. Arrows indicated cells with fragmented DNA.

respectively, compared to the control (4.3 ± 0.8%). An increase on HepG2 necrotic cells was observed for TP-07 32 μM (4.7 ± 0.4) and doxorubicin (88.7 ± 5.3) treatment compared to the control (0.9%) (Fig. 4.) Annexin V positive cells was not observed for HL60 cells, after 24 h of treatment with TAP-07 (1.6 μM) and TP-07 (12 μM) (data not shown).

3.2. In vivo assays

3.2.1. 2-Pyridyl 2,3-thiazole compounds reduce HepG2 cells development in xenograft model. To investigate whether the 2-pyridyl 2,3-thiazole compounds have in vivo antitumor activity, C·B-17 SCID mice were engrafted with HepG2 cells and treated with TAP-07 and TP-07 by

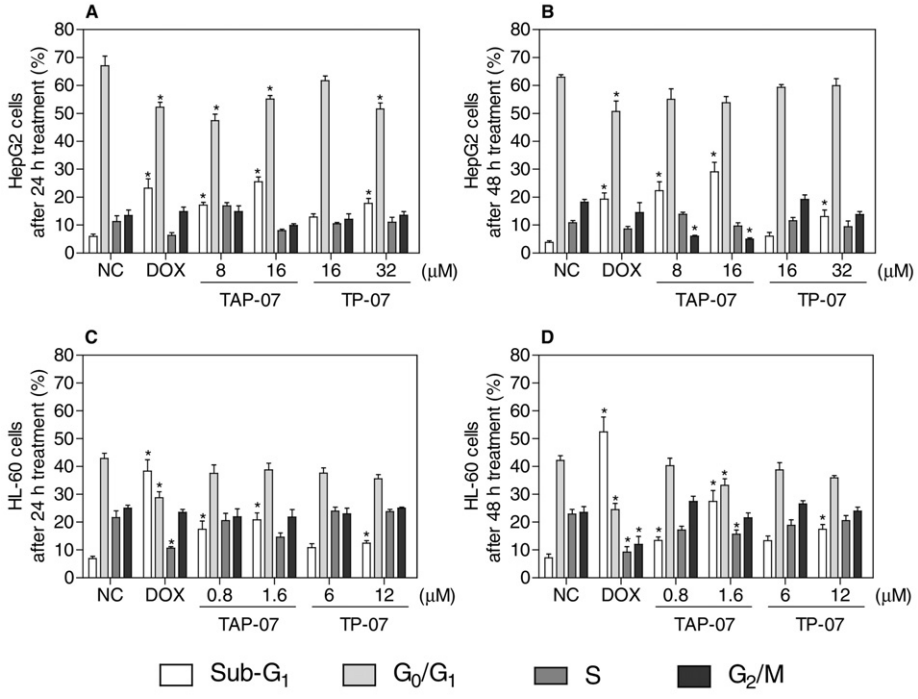


Fig. 3. DNA content analyzed by flow cytometry of HepG2 (A and B) and HL-60 (C and D) cells treated with TAP-07 and TP-07 after 24 (A and C) and 48 h (B and D) incubation. The negative control (NC) was treated with the vehicle (0.1% DMSO) used for diluting the substance tested. Doxorubicin (DOX, 2 μM) was used as the positive control. Data are presented as the mean ± S.E.M. of at the least three independent experiments performed in duplicate. Ten thousand events were evaluated per experiment and cellular debris was omitted from the analysis. *$p < 0.05$ compared with the negative control by ANOVA followed by Dunnett's Multiple Comparison Test.

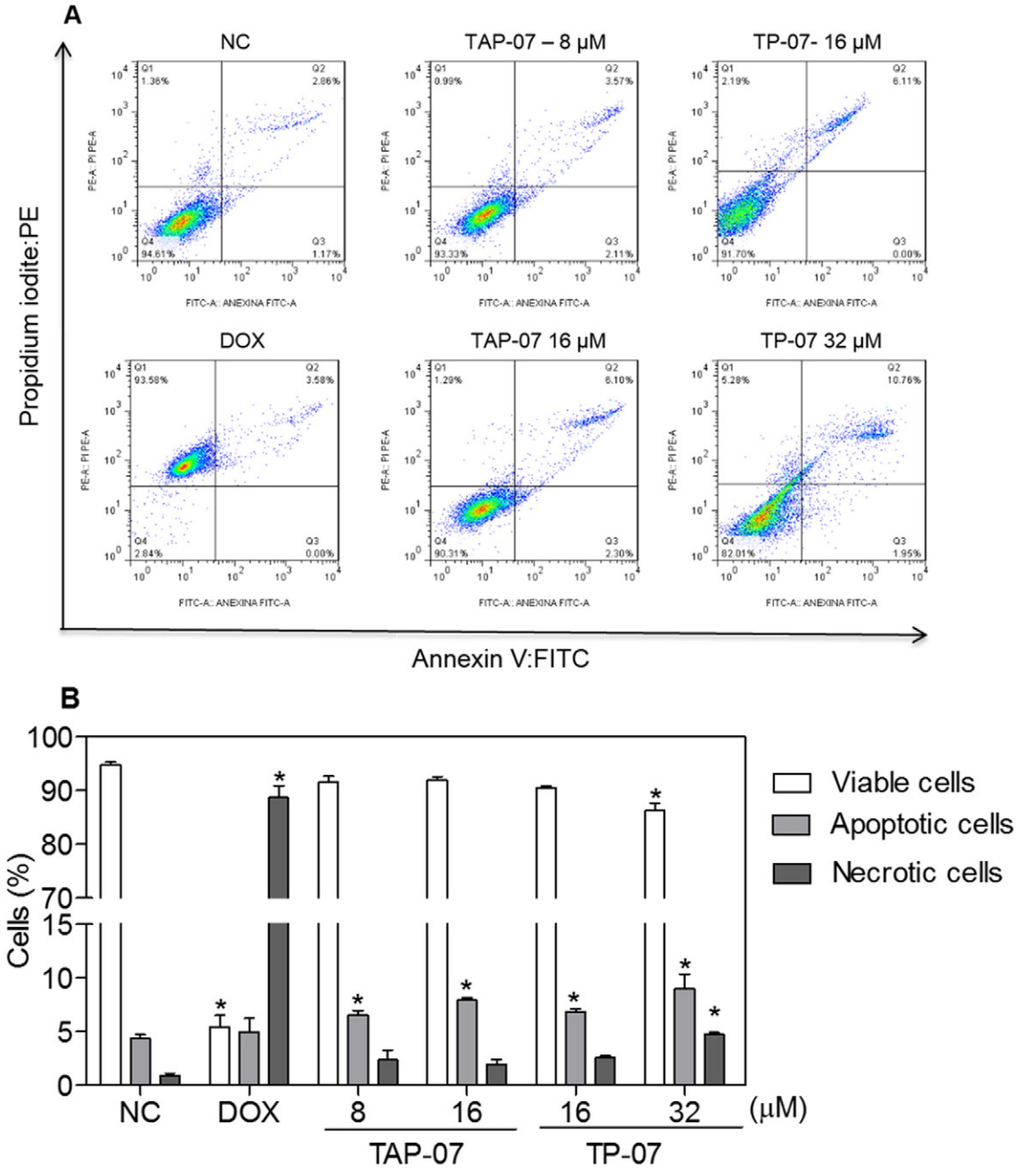


Fig. 4. Effect of TAP-07 and TP-07 compounds in the induction of apoptosis in HepG2 cell line after 24 h incubation. A – Representative flow cytometric dot plots showing the percentage of cells in viable, apoptotic and necrotic stages. B - Quantification of the cell viability determined by flow cytometry using annexin V/PI. The negative control (NC) was treated with the vehicle (0.1% DMSO) used for diluting the substance tested. Doxorubicin (DOX, 2 μM) was used as the positive control. Data are presented as the mean ± S.E.M. of at the least three independent experiments performed in duplicate. Ten thousand events were evaluated per experiment and cellular debris was omitted from the analysis. *$p < 0.05$ compared with the negative control by ANOVA followed by Dunnett's Multiple Comparison Test.

intraperitoneal route once a day for 21 consecutive days. Both TAP-07 and TP-07 were able to inhibit HepG2 cells growth in mice. Fig. 5A shows the inhibition obtained. On 22th day, the average tumor weight of the control mice was 0.60 ± 0.08 g. In the presence of TAP-07 and TP-07 (40 mg/kg), the average tumor weights were 0.19 ± 0.03 and 0.32 ± 0.04 g, respectively. Tumor mass was significantly reduced in TAP-07-treated animals by 73.0%, while treatment with TP-07 inhibited the tumor growth by 47.0%. The positive control (5-FU, 10 mg/kg) reduced the tumor weight by 41.4%. In histology analyses, we observed in all groups a hypervascularized solid pattern tumor showing polygonal tumor cells, displaying a pleomorphism, eosinophilic granular cytoplasm, rounded nuclei and prominent nucleoli. Mitotic figures, few pseudoglandular structures and steatotic areas were present. Tumoral cells were surrounded by fibrous stroma. Although necrosis was a frequent finding in all groups, this aspect was much more evident in TAP-07 group, when compared to the others, especially to the negative control (Fig. 5B)

Systemic toxicological parameters were examined in 2-pyridyl 2,3-thiazole compounds-treated mice. There was no difference on body weight of C·B-17 SCID mice bearing HepG2 cells tumor after 21 days of treatment with TAP-07 (40 mg/kg) or TP-07 (40 mg/kg), neither on liver, kidney, lung and heart wet weight, when compared to the negative control ($p > 0.05$) (Table 3). The liver weight of animals treated with the positive control (5-FU, 10 mg/kg) was reduced, when compared to the negative control ($p < 0.05$).

The animals were also analyzed regarding to the white and erythrocytes blood cells' counting (Table 4). It was found a decrease in the total

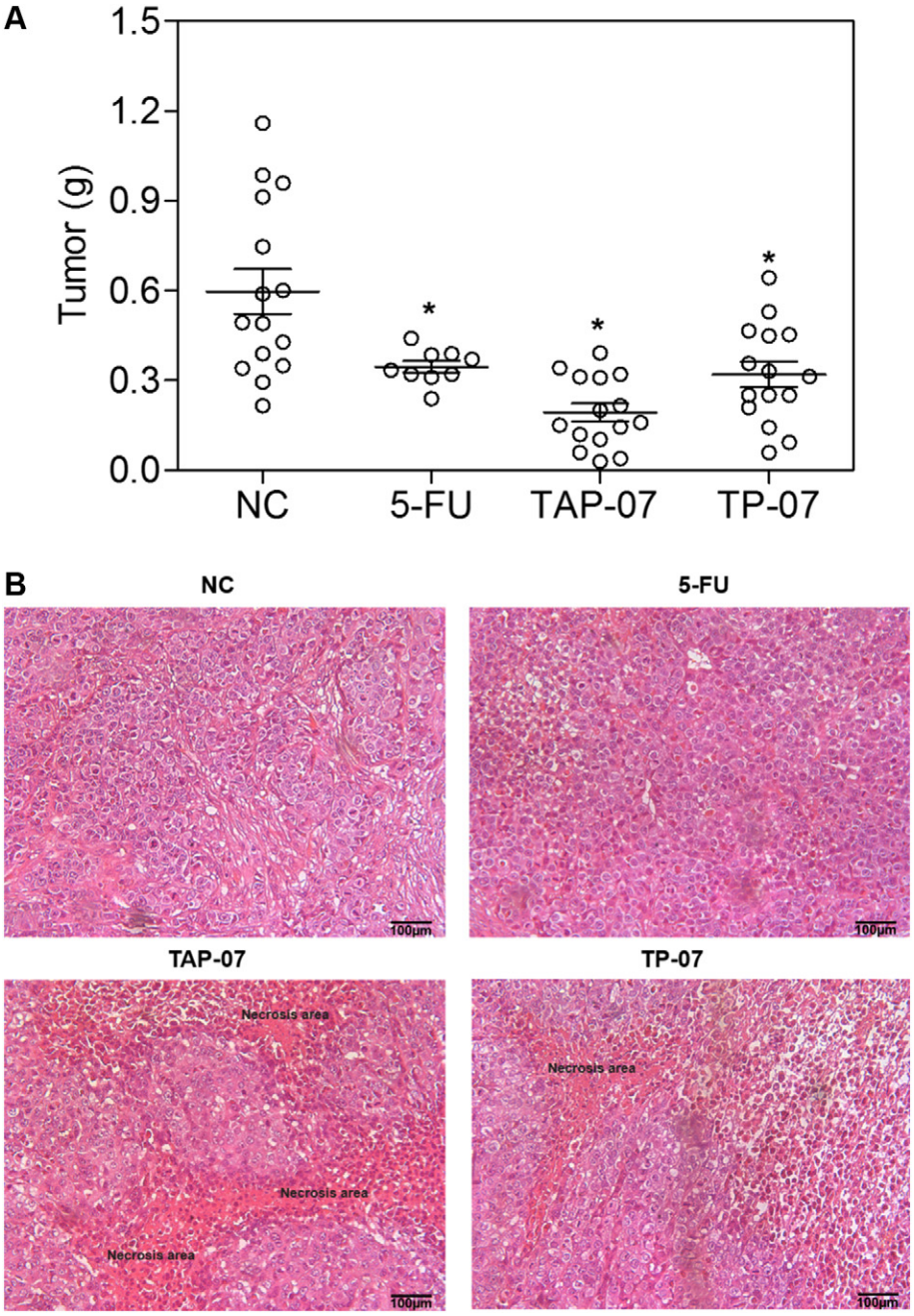


Fig. 5. In vivo antitumor activity of TAP-07 (40 mg/kg) and TP-07 (40 mg/kg) in C·B-17 SCID mice bearing HepG2 cells. A - Quantification of tumor weight. B - Representative histology analyses of the tumors. The negative control (NC) was treated with the vehicle (5% DMSO) used for diluting the substance tested. 5-Fluorouracil (5-FU, 10 mg/kg) was used as the positive control. Beginning 1 day after tumor implantation, the animals were treated through the intraperitoneal route for 21 consecutive days. Data are presented as the mean ± S.E.M. of 9–15 animals. $^{*}p < 0.05$ compared with the negative control by ANOVA followed by Dunnett's Multiple Comparison Test.

Table 3
Effect of TAP-07 and TP-07 on body and relative organ weight from C.B-17 SCID mice bearing HepG2 cells.

Parameters	NC	5-FU	TAP-07	TP-07
Dose (mg/kg)	–	10	40	40
Final body weight (g)	27.1 ± 1.3	26.1 ± 1.7	26.6 ± 1.6	26.9 ± 1.7
Initial body weight (g)	26.2 ± 1.9	26.2 ± 1.0	25.47 ± 1.9	26.9 ± 1.9
Liver (g/100 g body weight)	4.16 ± 0.8	3.37 ± 0.74*	3.65 ± 0.66	4.38 ± 1.0
Kidney (g/100 g body weight)	1.47 ± 0.37	1.15 ± 0.4	1.13 ± 0.38	1.59 ± 0.37
Heart (g/100 g body weight)	0.51 ± 0.19	0.50 ± 0.19	0.48 ± 0.10	0.46 ± 0.16
Lung (g/100 g body weight)	0.71 ± 0.29	0.62 ± 0.19	0.69 ± 0.20	0.61 ± 0.19

The negative control (NC) was treated with the vehicle (5% DMSO) used for diluting the substance tested. 5-Fluorouracil (5-FU, 10 mg/kg) was used as the positive control. Beginning 1 day after tumor implantation, the animals were treated through the intraperitoneal route for 21 consecutive days. Data are presented as the mean ± S.E.M. of 9–15 animals.

* $p < 0.05$ compared with the negative control (NC) by ANOVA followed by Dunnett's Multiple Comparison Test.

Table 4
Effect of TAP-07 and TP-07 on hematological parameters of peripheral blood from C.B-17 SCID mice bearing HepG2 cells.

Parameters	Non-tumor	NC	5-FU	TAP-07	TP-07
Dose (mg/kg)	–	–	10	40	40
Erythrocytes (10^6 cells/μL)	2.87 ± 0.6	3.32 ± 1.6*	2.2 ± 1.91*	2.17 ± 1.35*	2.15 ± 1.8*
Total leukocytes (10^3 cells/μL)	1.0 ± 0.2	5.3 ± 0.9	1.9 ± 0.4*	1.9 ± 0.8*	4.9 ± 1.1
Differential leukocytes (%)					
Neutrophil	41.0 ± 4.9	74.0 ± 6.9	65.0 ± 9.7	91.1 ± 2.2*	86.3 ± 3.8*
Lymphocyte	53.8 ± 5.3	22.0 ± 4.7	32.8 ± 9.3*	6.6 ± 2.3*	11.3 ± 3.0*
Monocyte	3.2 ± 0.7	1.0 ± 0	1.3 ± 0.5	1.0 ± 0	1.3 ± 0.8
Eosinophil	1.2 ± 0.4	1.3 ± 0.4	1.0 ± 0	1.1 ± 0.4	1.0 ± 0

Non-tumor group represents C.B-17 SCID mice without tumor inoculation or any treatment. The negative control (NC) was treated with the vehicle (5% DMSO) used for diluting the substance tested. 5-Fluorouracil (5-FU, 10 mg/kg) was used as the positive control. Beginning 1 day after tumor implantation, the animals were treated through the intraperitoneal route for 21 consecutive days. Data are presented as the mean ± S.E.M. of 6 animals.
* $p < 0.05$ compared with the negative control (NC) by ANOVA followed by Dunnett's Multiple Comparison Test.

number of leukocytes in TAP-07 and 5-FU groups compared to DMSO treated group ($p < 0.05$). If we compare the total leukocytes of TAP-07 treated animals (1.9 ± 0.4 10^3/μL) with the animals without tumor (1.0 ± 0.2 × 10^3/μL), no significant difference between groups is found. The erythrocytes number was also reduced in TAP-07, TP-07 and 5-FU groups.

The clinical biochemical parameters (Table 5) that were measured to investigate liver function alterations were alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin (ALB), cholesterol and total bilirubin. ALT, AST and ALP were significantly ($p < 0.05$) reduced after treatment with TAP-07 and TP-07 40 mg/Kg when compared to the control; nonetheless, the enzymes levels were at normal range. ALB, cholesterol and total bilirubin were not affected by the treatment. 5-FU did not change any biochemical parameters described above. As an indicator of kidney function, creatinine (CREA) and blood urea nitrogen (BUN) were measured. No alteration was found on BUN of animals treated with TAP-07, TP-07 and 5-FU, while for CREA levels, only animals treated with TP-07-40 mg/Kg presented a reduction on blood. Amylase, a pancreatic enzyme, was measured to verify pancreas damage. No alteration on blood amylase at any groups of animal treated with TAP-09, TP-07 and 5-FU was observed. Creatine Kinase with is correlated with cardiac and skeletal muscle lesion was reduced at all treated groups compared to the control ($p < 0.05$). Glucose, calcium and phosphate levels were not changed among the groups (Table 5).

Table 5
Effect of TAP-07 and TP-07 on clinical biochemical parameters of peripheral blood from C.B-17 SCID mice bearing HepG2 cells.

Parameters	NC	5-FU	TAP-07	TP-07
Dose (mg/kg)	–	10	40	40
ALT (U/L)	8.8 ± 2.1	10.2 ± 1.2	2.2 ± 0.5*	5.5 ± 0.5*
AST (U/L)	57.0 ± 3.7	64.0 ± 32.6	11.2 ± 3.2*	19.0 ± 2.1*
ALP (U/L)	49.6 ± 10.0	44.5 ± 6.7	12.0 ± 3.1*	11.7 ± 2.1*
ALB (mg/dl)	3.1 ± 0.4	3.0 ± 0.9	3.2 ± 0.6	1.8 ± 0.4
GLU (mg/dl)	203.0 ± 83.9	244.3 ± 45.2	281.0 ± 29.9	205.8 ± 4.4
CREA (mg/L)	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.08	0.3 ± 0.1*
CK (U/L)	77.6 ± 7.5	53.5 ± 2.3*	22.2 ± 3.3*	47.7 ± 1.7*
AML (U/ml)	1.243 ± 208	1.140 ± 444	1.078 ± 144	1.364 ± 261
Choleterol (mg/dl)	123.0 ± 33.1	195.5 ± 28.0	182.0 ± 12.8	72.0 ± 18.9
BUN (mg/dl)	45.6 ± 21.1	36.2 ± 7.2	33.9 ± 2.8	39.0 ± 8.9
BIL Total (mg/dl)	0.1 ± 0.05	0.1 ± 0.05	0.1 ± 0.05	0.2 ± 0.2
CALCIUM (mg/dl)	8.3 ± 1.3	8.6 ± 2.9	6.1 ± 0.7	6.9 ± 1.1
PHOSFATE (mg/dl)	16.7 ± 2.9	19.0 ± 1.0	17.3 ± 1.5	16.3 ± 0.7

The negative control (NC) was treated with the vehicle (5% DMSO) used for diluting the substance tested. 5-Fluorouracil (5-FU, 10 mg/kg) was used as the positive control. Beginning 1 day after tumor implantation, the animals were treated through the intraperitoneal route for 21 consecutive days. ALT: alanine aminotransferase. AST: aspartate aminotransferase; ALP: alkaline phosphatase; ALB: albumin; CREA: creatinine; CK: creatine kinase; AML: amylase; BUN: Blood urea nitrogen. BIL Total: Total bilirubin. Data are presented as the mean ± S.E.M. of 4–5 animals.
* $p < 0.05$ compared with the negative control by ANOVA followed by Dunnett's Multiple Comparison Test.

Morphological analysis was performed in the liver, kidneys, lungs and hearts of all groups (Fig. 6). Histopathological analysis of the livers revealed hydropic degeneration, dispersed areas of coagulation necrosis and inflammation in all experimental groups. It should be noted that these histopathological characteristics were more pronounced in the group TP-07, when compared to the other groups (negative control, 5-FU and TAP-07). In the kidneys, congestion, hemorrhage, glomerular sclerosis, tubular dilation and inflammation, as well as focal tubular necrosis at all groups were observed. In lungs, atelectasis, emphysema, focal hemorrhage, acute inflammation and vascular congestion were observed in all animals. Bronchiolar necrosis was observed in the groups TP-07 and TAP-07. Histopathological analysis of hearts did not present alterations in any group. Some histopathological features of this study (hydropic degeneration, vascular congestion and focal areas of inflammation) are acute cellular responses to the stimulus unrelated to the treatment and the injured cells can return to a homoeostatic state when the aggression ends.

4. Discussion

The in vitro cytotoxic activity and in vivo antitumor evaluation of TAP and TP compounds were studied for the first time at this communication. Most of the compounds were cytotoxic to at the least three cell lines, with exception to TP-11, which was cytotoxic to only one cell line (HL-60). The cytotoxicity of TAP and TP compounds at HL-60, MCF-7 and NCI-H292 did not changed significantly, being both groups of compounds highly potent. However, for HepG2 cell line, a few compounds presented cytotoxicity, being TAP-07 and TP-07 the most potent compounds.

Pyrydil 1,3-thiazole derivatives were described for presenting cytoxicity to HT29 colon cancer and Jurkat leukemia cells with IC_{50} values of 25.22 and 16.2 μM, respectively (dos Santos et al., 2016). In addition, pyridyl 1,3-thiazole derivatives induced TNF production on macrophages (dos Santos et al., 2016). Although being potent cytotoxic to tumor cells, some of TAP and TP compounds were also cytotoxic to non-tumor cells (PBMC) with resulting low selective index. Conversely, TAP-07 and TP-07 were not cytotoxic to non-tumor cells (PBMC) at concentrations tested and presented potent cytotoxicity to tumor cells growth. Due to selective **cytotoxicity** toward cancer cells, TAP-07 and TP-07 were selected for more experiments in two cell lines, HepG2 and HL-60.

Treatment with TAP-07 and TP-07 indicated reduction of the cell number, changes in morphological characteristics of the cells and increase in internucleosomal DNA fragmentation. These effects were observed for both HL-60 and HepG2 cells, being HL-60 more sensitive to both compounds than HepG2 cells. Apoptosis is characterized by externalization of phosphatidilserine, cytochrome c leakage from mitochondria and activation of caspases which initially leads to nuclear and cytoplasmatic condensation and ultimately the breaking up of the cell into a number of well-preserved fragments (Kerr et al., 1972); (Leist and Jäättelä, 2001). An increase in apoptotic HepG2 cells characterized

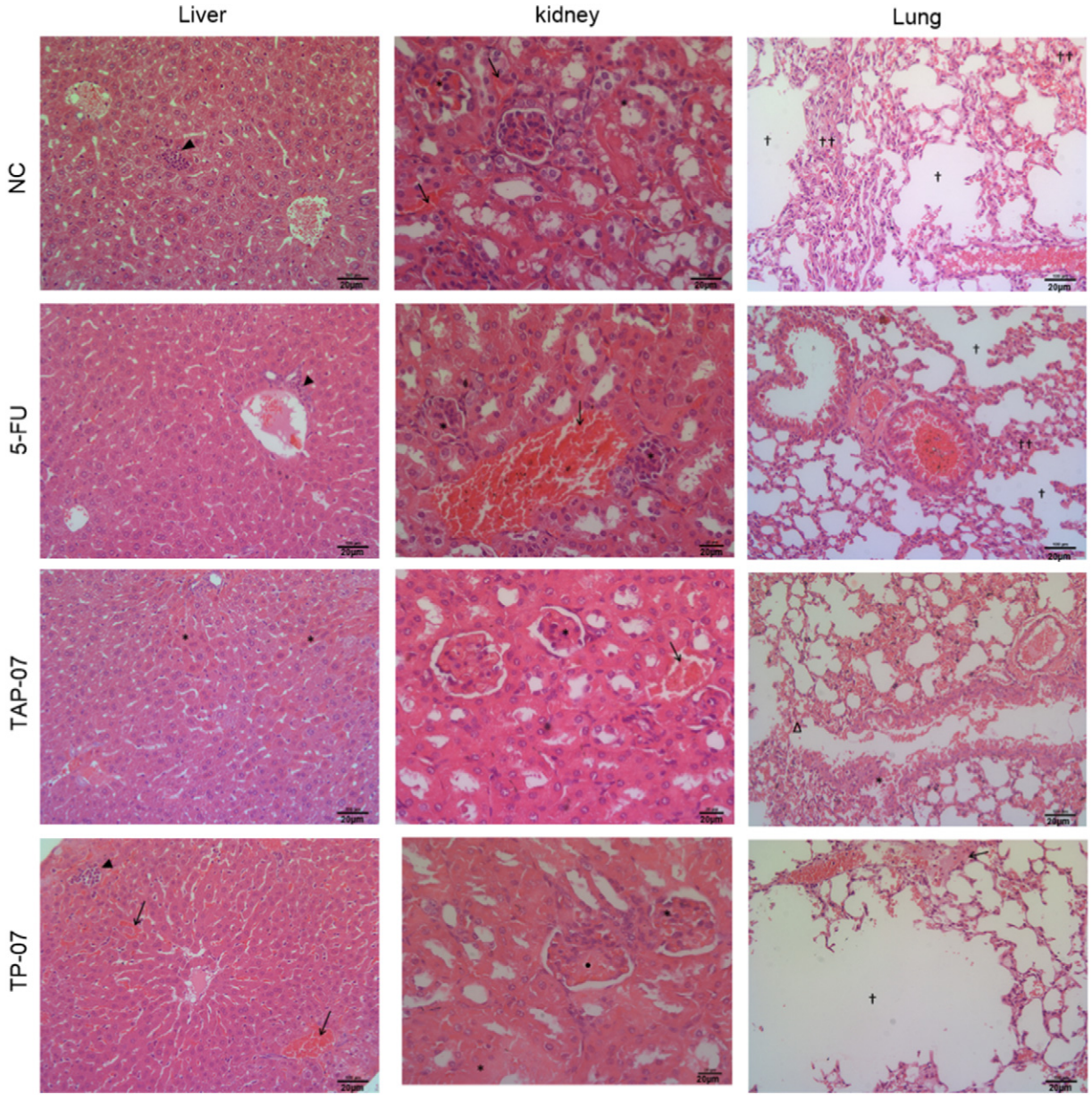


Fig. 6. Histopathological analysis of organs of C·B-17 SCID mice bearing HepG2 cells treated with TAP-07 (40 mg/kg) and TP-07 (40 mg/kg) stained with hematoxylin and eosin and analyzed by light microscopy. The negative control (NC) was treated with the vehicle (5% DMSO) used for diluting the substance tested. 5-Fluorouracil (5-FU, 10 mg/kg) was used as the positive control. Beginning 1 day after tumor implantation, the animals were treated through the intraperitoneal route for 21 consecutive days. Symbols: → congestion; ▲ inflammation; * necrosis; † emphysema; †† atalectasis; • hyalinization.

by externalization of phosphatidilserine was observed. However, the number of HL60 apoptotic cells was not increased after 24 h. Pyridyl-thiazoles compounds induced cell death by apoptosis in HT-29 cells after 72 h of treatment, while for Jurkat cell line a high level of propidium iodite labeled (necrosis) cells were observed (dos Santos et al., 2016). So far, TAP-07 was more potent than TP-07 in vitro cytotoxic evaluation. TAP-07 differs from TP-07 only by the methyl at C6. For trypanocidal activity, some features of the structure, such as the present of a methyl group at C6, the pyridine ring and the thiazole ring, were important (Cardoso et al., 2014).

Both TAP-07 and TP-07 were also tested in a hepatocellular carcinoma xenograft model. The tumors of animals treated with TAP-07 and TP-07 were reduced. In addition, necrosis area in the tumors was higher when animals were treated with TAP-07 compared to the others groups. In vivo tumor evaluation confirms the in vitro activity of pyridyl-thiazole compounds. It is worthwhile to emphasize that liver cancer is the second most common cause of death from cancer worldwide (Siegel et al., 2016; Theise, 2014).

Weight loss is a significant sign of toxicity on animals under treatment with cancer chemotherapy. Body weight gain and organ weigh of animals treated with TAP-07 and TP-07 was not different from the negative control suggesting the lack of severe toxicity. Liver enzymes were reduced after treatment with TAP-07 and TP-07. However, an increase on enzymes would suggest injure to the liver, rather than the reduction found (Giannini et al., 2005). Moreover, bilirrubin, albumin and cholesterol were at normal levels. The reduction of enzyme liver activity may be related to the presence of pharmacophores with known enzymatic inhibitory function. Molecules presenting a pyridine-thiosemicarbazones or its ciclization into thialoles as pharmacophores are described as enzymatic inhibitors. Triapine, a pyridine-thiosemicarbazone compound with anticancer properties, presents ribonucleotide reductase inhibitor activity (Niu et al., 1995). Some

hydrazine-thiazole derivatives displayed Human dihydroorotate dehydrogenase inhibition with in vivo anti-arthritic activity and rat liver mitochondrial monoamine oxidase inhibition (Li et al., 2015; Raciti et al., 1995) Kidney, lung and heart biochemical parameters were at normal range. The histopathological analysis of organs did not show any permanent lesion after treatment with pyrydil 1,3-thiazole derivatives. Total leukocytes were not reduced after treatment with TAP-07 when compared to animals without tumor. Leukocytosis is frequently found in patients with solid tumor and in some cases is associated to worse prognosis (Schernberg et al., 2017; Mabuchi et al., 2011). The treatment with TAP-07 brought back to the normal range the number of leukocytes, which were increased by presence of the tumor (DMSO-treated group). In fact, no symptoms of toxicity was perceive in animal treated with TAP-07 for 21 consecutive days. The development of new drugs with no or less side effect is a great challenge. Herein, we presented two novel compounds with anti-liver cancer activity and weak toxicity. In conclusion, TAP-07 and TP-07 presented anti-liver tumor effects both in vitro and in vivo with no major signs of toxicity, being TAP-07 which differs from TP-07 only by the methyl at C6, more potent as anti-tumor compound.

Funding

This work was financially supported by the Brazilian agencies CAPES, CNPq, FAPESB and FACEPE. (APQ-0213-2.10/13; AMD-0050-4.03/16)

Conflict of interest

The authors have declared that there is no conflict of interest.

Acknowledgment

The authors are grateful to the histotechnology and flow cytometry platforms of IGM/FIOCRUZ-Bahia for the performance of histological techniques and flow cytometric data acquisition, respectively.

References

Altaf, A.A., Shahzad, A., Gul, Z., Rasool, N., Badshah, A., Lal, B., Khan, E., 2015. A review on the medicinal importance of pyridine derivatives. 1, http://www.sciencepublishinggroup.com.1, doi:10.11648/J.JDDMC.20150101.11.

Beraldo, H., Gambinob, D., 2004. The wide pharmacological versatility of semicarbazones, thiosemicarbazones and their metal complexes. Mini-Rev. Med. Chem. 4:31–39. http://dx.doi.org/10.2174/1389557043487484.

Cardoso, M.V. de O., Siqueira, L.R.P. de, Silva, E.B. da, Costa, L.B., Hernandes, M.Z., Rabello, M.M., Ferreira, R.S., Cruz, L.F. da, Moreira, D.R.M., Pereira, V.R.A., Castro, M.C.A.B. de, Bernhardt, P.V., Leite, A.C.L., 2014. 2-Pyridyl thiazoles as novel anti-trypanosoma cruzi agents: structural design, synthesis and pharmacological evaluation. Eur. J. Med. Chem. 86:48–59. http://dx.doi.org/10.1016/j.ejmech.2014.08.012.

Niu, ChuanSheng Cheshire; Jun Li, Hamden; Xiuyan Li NeW, Haven; Terrence W. Doyle KillingWorth; Shu-Hui Chen, H., 1995. United States Patent [19] [11] Patent Number: [45] Date of Patent: doi:http://dx.doi.org/10.1074/JBC.274.42.30033.(51).

Egeblad, M., Werb, Z., 2002. New functions for the matrix metalloproteinases in cancer progression. Nat. Rev. Cancer 2:161–174. http://dx.doi.org/10.1038/nrc745.

Fortes, M.P., da Silva, P.B.N., da Silva, T.G., Kaufman, T.S., Militão, G.C.G., Silveira, C.C., 2016. Synthesis and preliminary evaluation of 3-thiocyanato-1H-indoles as potential anticancer agents. Eur. J. Med. Chem. 118:21–26. http://dx.doi.org/10.1016/j.ejmech.2016.04.039.

Giannini, E.G., Testa, R., Savarino, V., 2005. Liver enzyme alteration: a guide for clinicians. CMAJ 172:367–379. http://dx.doi.org/10.1503/cmaj.1040752.

Jansson, P.J., Kalinowski, D.S., Lane, D.J.R., Kovacevic, Z., Seebacher, N.A., Fouani, L., Sahni, S., Merlot, A.M., Richardson, D.R., 2015. The renaissance of polypharmacology in the development of anti-cancer therapeutics: inhibition of the "triad of death" in cancer by di-2-pyridylketone thiosemicarbazones. Pharmacol. Res. 100:255–260. http://dx.doi.org/10.1016/j.phrs.2015.08.013.

Kerr, J.F., Wyllie, A.H., Currie, A.R., 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer 26, 239–257.

Leist, M., Jäättelä, M., 2001. Four deaths and a funeral: from caspases to alternative mechanisms. Nat. Rev. Mol. Cell Biol. 2:589–598. http://dx.doi.org/10.1038/35085008.

Li, S., Luan, G., Ren, X., Song, W., Xu, L., Xu, M., Zhu, J., Dong, D., Diao, Y., Liu, X., Zhu, L., Wang, R., Zhao, Z., Xu, Y., Li, H., 2015. Rational design of benzylidenehydrazinyl-substituted thiazole derivatives as potent inhibitors of human dihydroorotate dehydrogenase with in vivo anti-arthritic activity. Sci Rep 5, 14836. http://dx.doi.org/10.1038/srep14836.

Mabuchi, S., Matsumoto, Y., Isohashi, F., Yoshioka, Y., Ohashi, H., Morii, E., Hamasaki, T., Aozasa, K., Mutch, D.G., Kimura, T., 2011. Pretreatment leukocytosis is an indicator of poor prognosis in patients with cervical cancer. Gynecol. Oncol. 122:25–32. http://dx.doi.org/10.1016/j.ygyno.2011.03.037.

Miwatashi, S., Arikawa, Y., Naruo, K.-I., Igaki, K., Watanabe, Y., Kimura, H., Kawamoto, T., Ohkawa, S., 2005. Synthesis and biological activities of 4-phenyl-5-pyridyl-1,3-thiazole derivatives as p38 MAP kinase inhibitors. Chem. Pharm. Bull. (Tokyo). 53, 410–418.

Morigi, R., Locatelli, A., Leoni, A., Rambaldi, M., 2015. Recent patents on Thiazole derivatives endowed with antitumor activity. Recent Pat. Anticancer. Drug Discov. 10, 280–297.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65:55–63. http://dx.doi.org/10.1016/0022-1759(83)90303-4.

Raciti, G., Mazzone, P., Raudino, A., Mazzone, G., Cambria, A., 1995. Inhibition of rat liver mitochondrial monoamine oxidase by hydrazine-thiazole derivatives: structure-activity relationships. Bioorg. Med. Chem. 3:1485–1491. http://dx.doi.org/10.1016/0968-0896(95)00137-6.

da S Maia, P.I., Pavan, F.R., Leite, C.Q.F., Lemos, S.S., de Sousa, G.F., Batista, A.A., Nascimento, O.R., Ellena, J., Castellano, E.E., Niquet, E., Deflon, V.M., 2009. Vanadium complexes with thiosemicarbazones: synthesis, characterization, crystal structures and anti-mycobacterium tuberculosis activity. Polyhedron 28:398–406. http://dx.doi.org/10.1016/j.poly.2008.11.017.

dos Santos, T.A.R., da Silva, A.C., Silva, E.B., Gomes, P.A.T. de M., Espíndola, J.W.P., Cardoso, M.V. de O., Moreira, D.R.M., Leite, A.C.L., Pereira, V.R.A., 2016. Antitumor and immunomodulatory activities of thiosemicarbazones and 1,3-Thiazoles in Jurkat and HT-29 cells. Biomed Pharmacother 82:555–560. http://dx.doi.org/10.1016/j.biopha.2016.05.038.

Schernberg, A., Escande, A., Rivin Del Campo, E., Ducreux, M., Nguyen, F., Goere, D., Chargari, C., Deutsch, E., 2017. Leukocytosis and neutrophilia predicts outcome in anal cancer. Radiother. Oncol. 122 (1):137–145. http://dx.doi.org/10.1016/j.radonc.2016.12.009.

Siegel, R.L., Miller, K.D., Jemal, A., 2016. Cancer statistics, 2016. CA Cancer J. Clin. 66:7–30. http://dx.doi.org/10.3322/caac.21332.

da Silva, J.B.P., Navarro, D.M. do A.F., da Silva, A.G., Santos, G.K.N., Dutra, K.A., Moreira, D.R., Ramos, M.N., Espíndola, J.W.P., de Oliveira, A.D.T., Brondani, D.J., Leite, A.C.L., Hernandes, M.Z., Pereira, V.R.A., da Rocha, L.F., de Castro, M.C.A.B., de Oliveira, B.C., Lan, Q., Merz, K.M., 2015. Thiosemicarbazones as *Aedes aegypti* larvicidal. Eur. J. Med. Chem. 100:162–175. http://dx.doi.org/10.1016/j.ejmech.2015.04.061.

Theise, N.D., 2014. Liver Cancer. In: Stewart, B.W., Wild, C.P. (Eds.), World Cancer Report. International Agency for Research on Cancer, Distributed by World Health Organization, pp. 403–412.

Zhang, Y., Tounge, B.A., Wang, A. Hawkins, M., Leonard, K.A., Barbay, J.K., Maharoof, U.S.M. 2011b. Pyridyl-thiazolyl inhibitors of pro-matrix metalloproteinase activation. WO 2012068210A1. http://www.google.com.na/patents/wo20120168210A1?cl=en&hl=pt-BR/(accessed 13.01.17).