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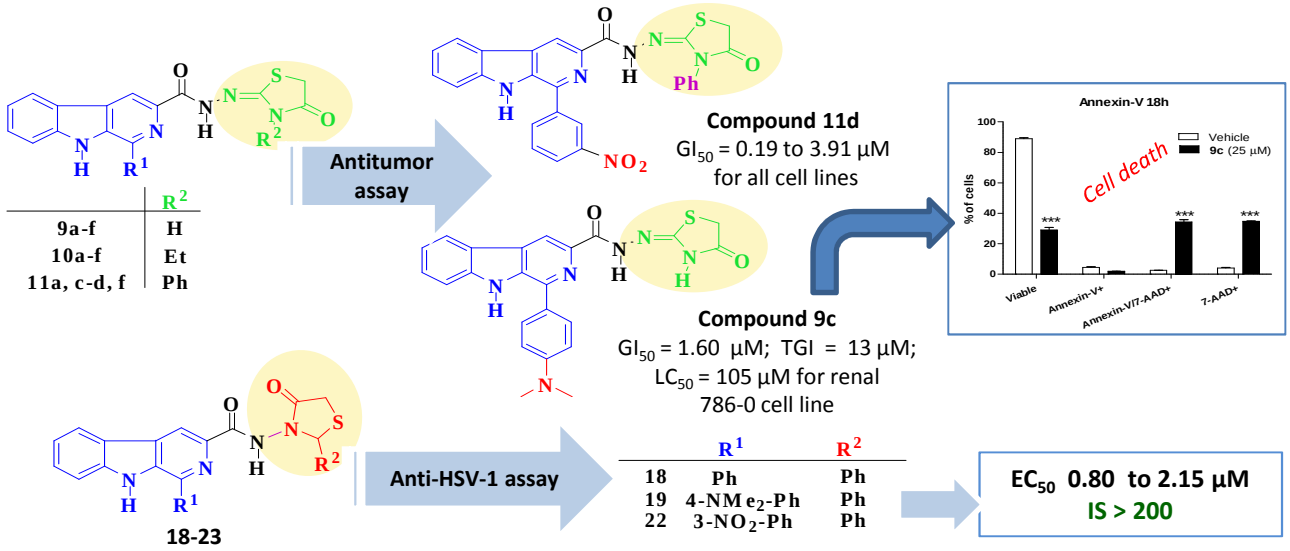

Antitumor assay
Compound 11d
GI50 = 0.19 to 3.91 µM
for all cell lines
Compound 9c
GI50 = 1.60 µM; TGI = 13 µM;
LC50 = 105 µM for renal
786-0 cell line
9a-f H
10a-f Et
11a, c-d, f Ph
Anti-HSV-1 assay
18-23
18 Ph Ph
19 4-NMe2-Ph Ph
22 3-NO2-Ph Ph
EC50 0.80 to 2.15 µM
IS > 200
Annexin-V 18h
Vehicle
9c (25 µM)
Cell death
% of cells
Viable
Annexin-V+
Annexin-V/7-AAD+
7-AAD+

Synthesis and evaluation of novel hybrids *β*-carboline-4-thiazolidinones as potential antitumor and antiviral agents

Valéria Aquilino Barbosa[a], Paula Baréa[a], Renata Sespede Mazia[b], Tania Ueda-Nakamura[b], Willian Ferreira da Costa[a], Mary Ann Foglio[c], Ana Lucia T. Goes Ruiz[c], João Ernesto de Carvalho[c], Débora Barbosa Vendramini Costa[c], Celso Vataru Nakamura[b] and Maria Helena Sarragiotto[a,*]

[a] Universidade Estadual de Maringá (UEM), Departamento de Química, Av. Colombo, 5790, CEP: 87020-900 Maringá, PR, Brazil

[b] Universidade Estadual de Maringá (UEM), Departamento de Análises Clínicas, Universidade Estadual de Maringá, Av. Colombo, 5790, CEP: 87020-900 Maringá, PR, Brazil

[c] Universidade Estadual de Campinas (Unicamp), Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA), CEP: 13083-970 Campinas, SP, Brazil

*corresponding author. E-mail: mhsarragiotto@uem.br (M. H. Sarrragiotto)

ABSTRACT

A series of novel hybrids *β*-carboline-4-thiazolidinones were synthesized and evaluated for their *in vitro* antitumor activity against human cancer cell lines and for antiviral activity towards *Herpes Simplex* virus type-1 (HSV-1). From the *N'*-(2-ylidene-4-thiazolidinone)-*β*-carboline-3-carbohydrazide series (**9-11**), **c**ompounds **9c** and **11d** were the most active, showing growth inhibition 50% (GI_{50}) values less than 5 µM for all cell lines tested. Compound **9c**, bearing the 4-dimethylaminophenyl group at C-1 of *β*-carboline was selected for further investigation concerning cell death and cell cycle profile, focusing on the human renal adenocarcinoma cell line 786-0. Treatments with 25 µM of compound **9c** induced cell death after 15 h of treatment, characterized by phosphatidylserine exposure and loss of membrane integrity. Moreover, treatment with 12.5 µM promoted a sub-G1 arrest, which indicates cell death. Derivatives of the *N*-(2-substituted-aryl-4-thiazolidinone)-*β*-carboline-3-carboxamide series (**18-23**) showed a potent activity and high selectivity for glioma (U251) and ovarian (OVCAR-3) cancer cell lines. Also, some *β*-carboline-4-thiazolidinone hybrids showed potent antiviral activity against *Herpes simplex* virus type-1. The *N*-(2-substituted-aryl-4-thiazolidinone)-carboxamide moiety in **18**, **19** and **22** confer a potent anti-HSV-1 activity for these derivatives, which presented EC_{50} values of 0.80, 2.15 and 2.02 µM, respectively. The assay results showed that the nature of 4-thiazolidinone moiety and of the substituent attached at the 3- and 1- position of *β*-carboline nucleus, respectively, influenced the antitumor and antiviral activities.

Keywords: *β*-carboline, 4-thiazolidinone, antitumor, antiviral, medicinal chemistry

1. Introduction

Naturally occurring and synthetic *β*-carboline alkaloids are widely studied due to their large spectrum of important pharmacological and biological properties, such as antitrypanosomal and antileishmanial [1-4], anti-Alzheimer [5,6], anti-platelet aggregation and anti-thrombotic [7,8], anti-Parkinson [9], and as DYRK1A inhibitors [10,11]. It's worth mentioning the potent antitumor activity of this class of compounds that has been reported in several studies involving design, synthesis and structure-activity relationship (SAR) of *β*-carboline derivatives [12-20]. These studies revealed that the introduction of appropriate substituents into 1-, 3- and 9-positions of *β*-carboline ring can result in more potent drugs with reduced toxicity and neurotoxic effects. Besides the antitumor activity, recent reports have been shown that β-carboline derivatives can act as antiviral agents against Human Immunodeficiency Virus type 1 (HIV-1) and type 2 (HIV-2) [21-23], and Tobacco Mosaic Virus (TMV) [24]. Also, recent works showed that several nitrogen heterocyclic compounds displayed anti-HSV activity [25-28].

In our previous work we demonstrated that the presence of an appropriately substituted phenyl group at 1-position, and substituents at 3-position of the *β*-carboline nucleus, enhanced the antitumor activity [29-33]. Also, our work indicated that hybrids of *β*-carboline with different heterocyclic nucleus at 3-position, as for compounds **I, II** and **III** (**Fig. 1**), displayed potent antitumor properties [30, 32, 33]. In addition, the interaction of compound **I** with DNA was investigated by using UV and fluorescence spectroscopic analysis. Our studies showed that **I** interact with ctDNA by intercalation binding [30].

The antiviral activity of 1-(substituted-phenyl)-N'-(substituted-benzylidene)-*β*-carboline-3-carbohydrazides against HSV-1 and vaccinal poliovirus (VP) was also demonstrated for our research group [34, 35].

4-Thiazolidinone derivatives have been extensively investigated due to their gamma of biological activities, such as anti-inflammatory, anticonvulsant, anti-diabetic, cardiovascular, anti-tubercular, antifungal, antibacterial, antiviral, as well as anticancer activity against different cell lines [36-38].

Concerning to anticancer activity, recent reports have indicated that 4-thiazolidinones can inhibit tumor cell proliferation through inducing cell cycle arrest [39-48]. This was demonstrated for a series of 2,3-diaryl-4-thiazolidinone derivatives,

especially for compound **IV** (**Fig. 1**), which displayed potent inhibitory effects on the proliferation of A549 (human lung cancer) and MDA-MB-231 (human breast cancer) [39]. Also, new hybrids acridine–thiazolidinone (compounds **V**, **Fig. 1**), displaying strong cytotoxic activity *in vitro* against leukemic cells HL-60 and L1210, and human epithelial ovarian cancer cell lines A2780, inhibited cells proliferation and induced an arrest of the cell cycle and cell death. The effects on cells were associated with their reactivity towards thiols and DNA binding interaction [40].

A compound from the 2-(thienothiazolylimino)-1,3-thiazolidin-4-ones series synthesized by Huber-Villaume et al. [41] was found to be potential inhibitor of CDC25 protein, with an IC_{50} of 6.2 ± 1.0 µM. Treatment with the active 4-thiazolidinone derivative led to MCF7 and MDA-MB-231 cell growth arrest. Studies developed by Zhang et al. [48] shown that compounds bearing thiazolidinone nucleus act as either microtubule polymerization inhibitors or histone deacetylase inhibitors. The derivatives act synergistically, targeting multiple proteins and leading to the regulation of cell cycle checkpoint proteins, which resulted in the G2/M cell cycle arrest and cell apoptosis [42].

The properties of 4-thiazolidinones to inhibit proliferation through inducing cell cycle arrest action, and through other action mechanisms, as well as the potent anticancer exerted for 4-thiazolidinone derivatives, led us to explore this scaffold to search for potential agents for treating multi-factorial diseases such cancer. The molecular hybridization of *β*-carboline and 4-thiazolidinone pharmacophores was chosen as a strategy to obtain more active compounds that impact multiple anticancer targets [43]. Furthermore, as both *β*-carboline and 4-thiazolidinone nucleus exhibit antiviral activity, it is expected that the proposed hybrids are also active against HSV-1.

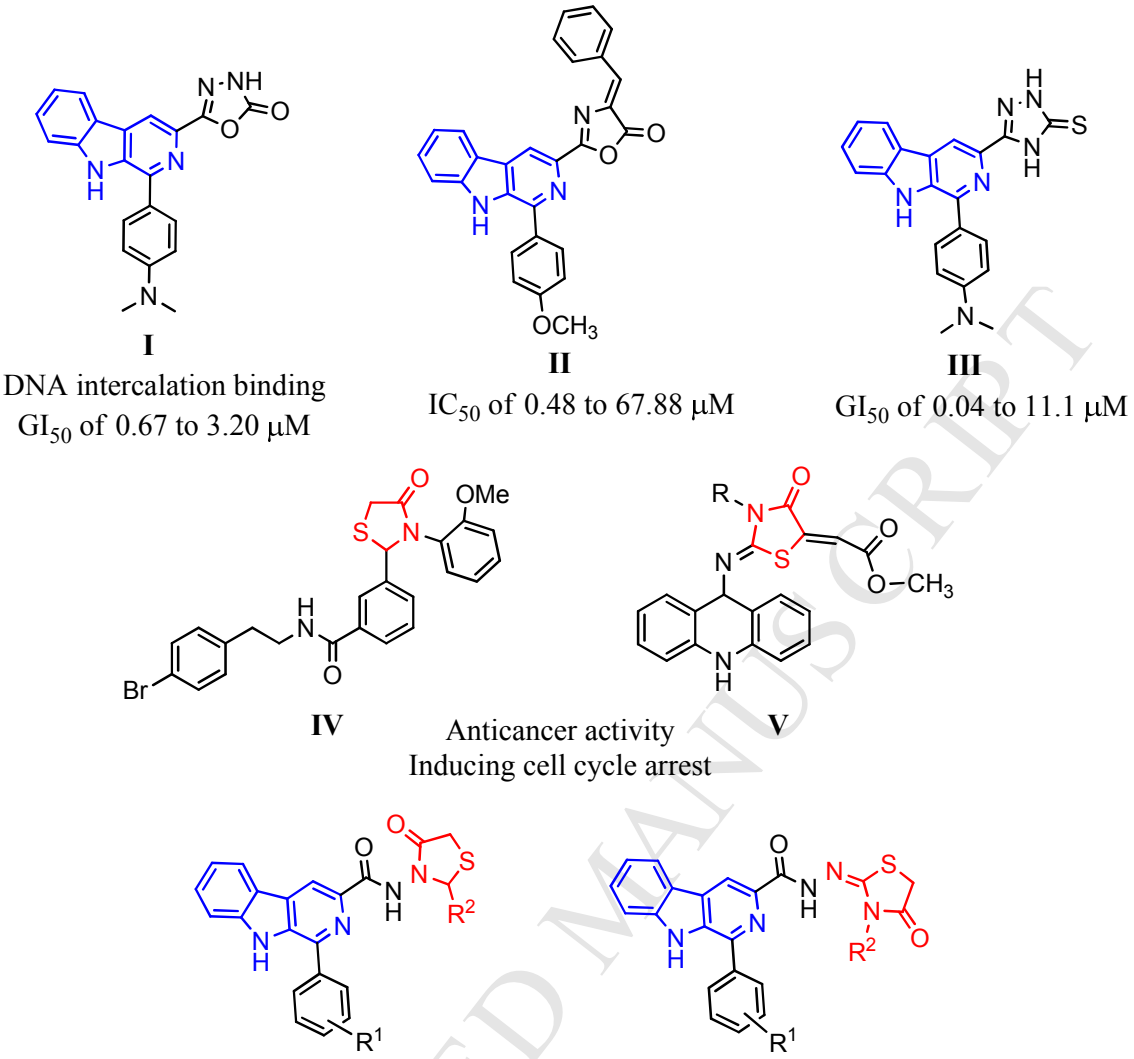


Figure 1: *β*-Carboline (**I-III**) and thiazolidinone (**IV** and **V**) derivatives with anticancer activity.

More specifically, in this work we describe the synthesis of a series of *N'*-(2-ylidene-4-thiazolidinone)-*β*-carboline-3-carbohydrazides and *N*-(2-substituted-aryl-4-thiazolidinone)-*β*-carboline-3-carboxamides, as well as the antiviral activity against *Herpes simplex* virus (HSV-1), and antitumor activity towards human cancer cell lines of the new synthetized compounds. Additionally, studies evaluating cell death and influence in the cell cycle profile were also carried out for the active compound **9c**, focusing on the human renal adenocarcinoma cell line 786-0.

2. Results and discussion

2.1. *Chemistry*

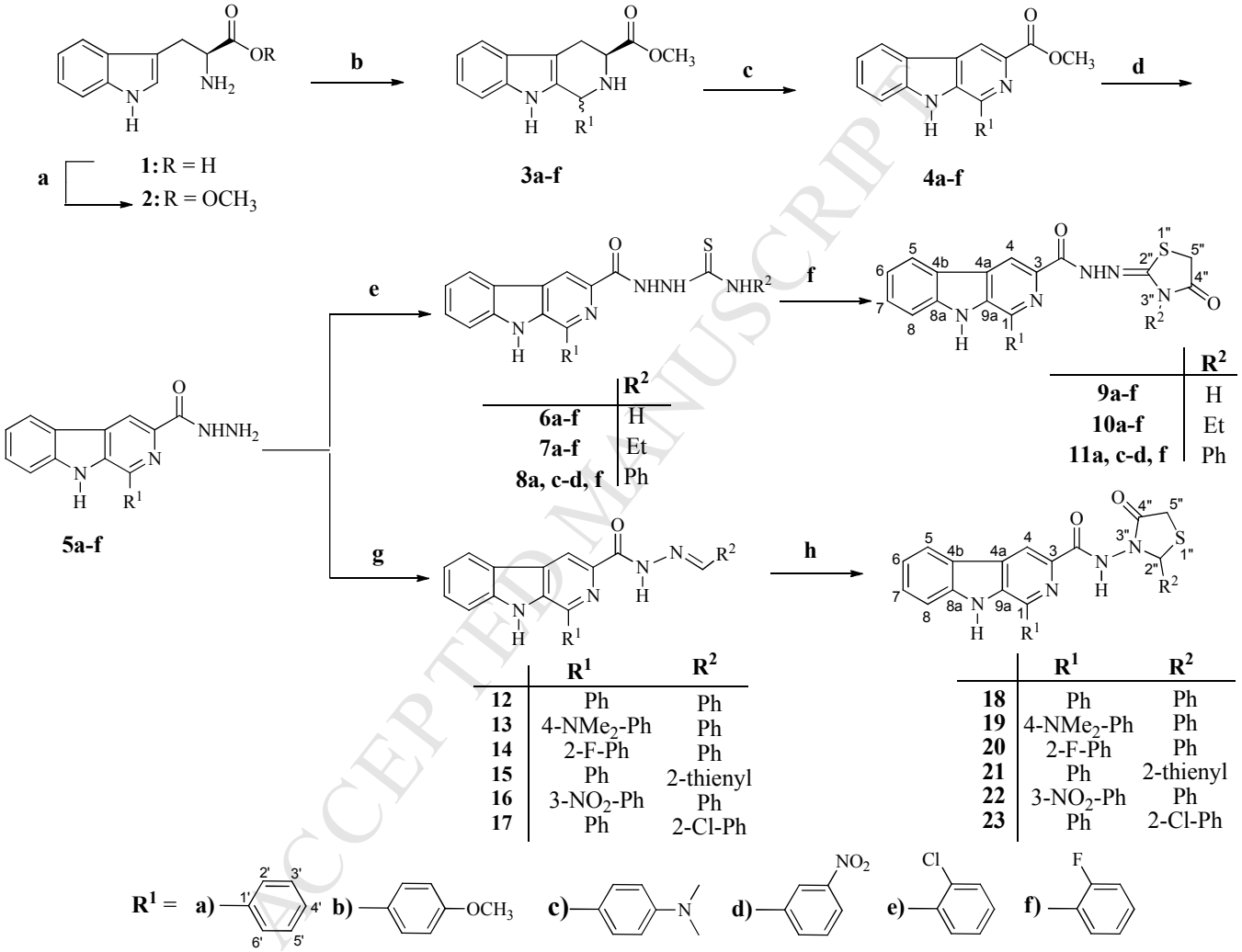
The synthetic routes employed in the preparation of the hybrids *N'*-(2-ylidene-4-thiazolidinone)-*β*-carboline-3-carbohydrazides **(9a-f), (10a-f)** and **(11a, c-d, f),** and of *N*-(2-substituted-4-thiazolidinone)-*β*-carboline-3-carboxamides **(18-23)** are shown in **Scheme 1.**

To obtain the hybrids *β*-carbolines-4-thiazolidinones proposed, firstly the *β*-carboline-3-carbohydrazides (**5a-f**), the common intermediates for the synthetic routes, were synthetized. For this, the commercial *L*-tryptophan **(1)** was esterified with methanol in the presence of H_2SO_4 to afford *L*-tryptophan methyl ester (**2**), which was subjected to Pictet Spengler condensation reaction with different aromatic aldehydes, in acidic media, to provide the tetrahydro-*β*-carboline-3-carboxylates (**3a-f**). The oxidation of **3a-f** with sulfur in xylene under reflux afforded the *β*-carbolines **4a-f**. The *β*-carboline-3-carbohydrazides (**5a-f**) were obtained by nucleophilic substitution reaction of the *β*-carbolines **4a-f** with hydrazine hydrate in ethanol.

The synthesis of *N'*-(2-ylidene-4-thiazolidinone)-*β*-carboline-3-carbohydrazides (**9a-f**) was carried out by the reaction of *β*-carboline-3-carbohydrazides (**5a-f**) with potassium isothiocyanate, in ethanol, under reflux, followed by condensation reaction of thiosemicarbazides **6a-f** with ethyl bromoacetate, using sodium acetate and potassium hydroxide in ethanol.

To evaluate the effect of a substituent at the nitrogen of thiazolidinone ring on activity, the carbohydrazides **5a-f** were treated with ethyl isothiocyanate and phenyl isothiocyanate to give the respective thiosemicarbazides **7a- f** and **8a, 8c-d** and **8f**, which afforded the *N*-substituted derivatives **10a- f** and **11a, 11c-d** and **11f** by using the reaction conditions for preparation of derivatives **9a-f**.

To obtain the *N*-(2-substituted-aryl-4-thiazolidinone)-*β*-carboline-3-carboxamides (**18-23**) the *β*-carboline-3-carbohydrazides **5**(**a, c, d, f**) were subjected to reaction with aromatic aldehydes in DMF under microwave irradiation to afford *β*-carbolines-3-benzylidenecarbohydrazides (**12-17**). The formation of *N*-(2-substituted-aryl-4-thiazolidinone)-*β*-carboline-3-carboxamides (**18-23**) was possible with addition of the catalytic amount of *p*-toluenesulfonic acid to the reaction of imine intermediates **12-17** and thioglycolic acid, under toluene reflux.



Scheme 1. Reagents and conditions: (a) MeOH, H_2SO_4 (*cat*), reflux, 48 h, (b) R^1CHO, TFA, DCM, rt; (c) S, xylene, reflux, 48 h to 0 °C, 3 h; (d) $NH_2NH_2.H_2O$, EtOH, reflux, 48 h, 77%: (e) KSCN, R^2NCS, EtOH, reflux; (f) $BrCH_2CO_2C_2H_5$, AcONa, KOH, EtOH reflux; (g) DMF, R^2CHO, MW; (h) $HSCH_2COOH$, *p*-TsOH (*cat*), toluene, reflux.

The sythetized compounds were characterized by their spectral data (HRMS-ESI, ^{1}H NMR and ^{13}C NMR). The characterization of the *N'*-(2-ylidene-4-thiazolidinone)-*β*-carboline-3-carbohydrazides without substituent at the nitrogen of thiazolidinone ring **(9a-f)** was mainly supported by signals at δ_H 3.52 – 4.05 (*s*, 2H, C$\underline{H}_2$, H-5") in ^{1}H NMR spectra, and signals at δ_C 34.2 – 38.0 (C$\underline{H}_2$, C-5"), δ_C 172.1 – 182.4 (C=O, C-4"), δ_C 162.7 – 167.3 (C=O, carbohydrazide) and δ_C 150.8 – 162.7 (C=N, C-2") in the ^{13}C NMR spectra.

The *N'*-(3-ethyl-2-ylidene-4-thiazolidinone)-*β*-carboline-3-carbohydrazides (**10a-f**) were characterized by signals at δ_H 3.86 – 4.19 (*s* or *m*, 2H C$\underline{H}_2$, H-5"), δ_H 3.76 – 4.00 (*m* or *quart*. *J* = 6.1 – 7.0 Hz; 2H $CH_3C\underline{H}_2$), δ_H 1.20 – 1.33 (*t*, *J* = 6.3 – 7.2 Hz; 3H $C\underline{H}_3CH_2$) in the ^{1}H NMR spectra. Signals at δ_C 29.6 – 38.7 (C$\underline{H}_2$, C-5"), δ_C 32.9 – 38.9 ($CH_3\underline{C}H_2$), δ_C12.1 – 14.5 ($\underline{C}H_3CH_2$), δ_C 170.7 – 171.5 (C=O, C-4"); δ_C 159.6 – 162.7 (C=O, carbohydrazide) and δ_C 151.1 – 161.9 (C=N, C-2") in the ^{13}C NMR spectra confirmed the formation of these products.

The formation of *N'*-(3-phenyl-2-ylidene-4-thiazolidinone)-*β*-carboline-3-carbohydrazides **11**(**a, c-d, f**) was evidenced by the signals at δ_H 3.04 – 4.33 (*s*, 2H, C$\underline{H}_2$, H-5") and by the hydrogens signals of the phenyl group attached to the nitrogen atom. In the ^{13}C NMR spectra were observed signals at δ_C 33.0 – 33.2 (C$\underline{H}_2$, C-5"), δ_C 170.5 – 171.2 (C=O, C-4"), δ_C 158.3 –160.5 (C=O, carbohydrazide), and δ_C 159.1 – 160.3 (C=N, C-2").

The *N*-(2-substituted-aryl-4-thiazolidinone)-*β*-carboline-3-carboxamides (**18-23**) were characterized by the signals at δ_H 3.78 – 3.84 (*dd*, *J* = 1.5 – 1.8 and 15.0 Hz, 1H C$\underline{H}_2$, H_b-5") coupled in W with hydrogen (C$\underline{H}$, H-2") of thiazolidinone ring, δ_H 3.92 – 4.00 (*d*, *J* = 15.0 – 15.9, 1H, C$\underline{H}_2$, H_a-5"), δ_H 6.01 – 6.57 (*s*, 1H, C$\underline{H}$, H-2") in the ^{1}H NMR spectra. In the ^{13}C NMR spectra were observed signals at δ_C 29.5 – 30.5 (C$\underline{H}_2$, C-5"), δ_C 60.2 – 63.7 (C$\underline{H}$, C-2"), δ_C 169.2 – 170.9 (C=O, C-4"), and δ_C 162.9 – 165.1 (C=O, carboxamide).

2.2. Biological activities

2.2.1. Antitumor activity

The *in vitro* anticancer activities of the synthesized *β*-carboline-4-thiazolidinones were evaluated against nine human tumor cell lines – U251 (Glioma), UACC-62 (melanoma), MCF-7 (Breast), NCI/ADR-RES (ovarian expressing multiple-

drug-resistance phenotype), 786-0 (Renal), NCI-H460 (Lung), PC-3 (Prostate), OVCAR-3 (Ovarian) and HT-29 (Colon). The response parameter GI_{50} was calculated for each compound and cell line tested, and the results were summarized in **Table 1** and **2**. The GI_{50} values (growth inhibitory activity) refer to the drug concentration that inhibits in 50% the cellular growth when compared to untreated control cells. Compounds with GI_{50} values ≥ 100 μM were considered not active.

The analysis of GI_{50} values (**Table 1**) showed that for the *N'*-(2-ylidene-4-thiazolidinone)-*β*-carboline-3-carbohydrazides (**9a-f**), the compound **9a** containing phenyl group in the 1-position of the *β*-carboline ring was active for all human tumor cell lines tested, with GI_{50} values less than 30 μM.

In order to verify the influence of electron-withdrawing and -donating groups in antitumor activity, the phenyl ring with different groups (OCH_3, NMe_2, NO_2, Cl and F) was introduced in the 1-position the *β*-carboline skeleton.

The substitution of the phenyl group at C-1 in **9a** for a 4-methoxyphenyl group (**9b**) or 4-dimethylaminophenyl group (**9c**) led to an improvement of activity for most of cell lines tested. On the other hand, the presence of the withdrawing nitro substituent at the phenyl group resulted in loss of antitumor activity, being the derivative **9d** inactive towards lung (NCI-H460), ovarian (OVCAR-3), and colon **(**HT-29) cell lines. However, a potent activity (GI_{50} = 0.62 μM) and selectivity was observed for this derivative against NCI/ADR-RES (ovarian expressing multiple-drug-resistance phenotype). Selectivity was also observed for the derivative **9e**, which contains 2-chlorophenyl group in C-1, presenting a GI_{50} value of 0.01 μM against ovarian (OVCAR-3) cells.

To investigate the effect of substituents at the nitrogen atom of 4-thiazolidinone ring, *N*-substituted derivatives **10a- f** and **11(a, c-d, f)**, bearing the N^3-ethyl and N^3-phenyl groups, respectively, were also evaluated for their antitumor activity.

In general, the introduction of the N^3-ethyl group did not contribute to the overall increase in activity, but enhanced the selectivity of some of the derivatives against specific tumor cells. The derivative **10a** was selective for glioma (U251) with GI_{50} value of 0.46 μM. The derivative **10e** was selective for glioma and ovarian expressing multiple-drug-resistance phenotype (NCI/ADR-RES), with GI_{50} values of 0.74 and 0.25 μM, respectively. Strong selectivity was also observed for the derivative **10d** against NCI/ADR-RES, which presented GI_{50} value of 3.27 μM for this cancer cell lines, and GI_{50} values greater than 100 μM for all other cells tested.

On the other hand, the presence of a phenyl group at the 3-position of thiazolidinone ring provide an enhancement of activity for the derivative containing the 4-nitrophenyl at C-1 (**11d**), compared to their analogs **9d** and **10d**. Compound **11d** was the most active of the *N'*-(2-ylidene-4-thiazolidinone)-*β*-carboline-3-carbohydrazide series, showing GI_{50} values in the range of 0.83 to 3.81 μM for all cell lines tested.

Comparison of GI_{50} values (**Table 1**) of the most active derivatives **9c** and **11d** with those of compound **I** (**Fig. 1**) described previously for our group [30] reveled that these compounds displayed similar activity. On the other hand, the incorporation of 4-thiazolidinone nucleus into *β*-carboline skeleton resulted in an enhancement of antitumor activity for **9c** and **11d** compared to compounds **II** and **III** [31, 33] (**Fig 1**), as expected.

Table 1. *In vitro* antitumor activity (GI_{50} in µM) of 1-(substituted-phenyl)-*β*-carboline 3-(substituted-4-thiazolidinone) derivatives **9-11**.

			Cell lines						
Comp	R^1	R^2	Glioma (U251)	Breast (MCF-7)	Ovarian Resistant (NCI/ADR-RES)	Renal (786-0)	Lung (NCI-H460)	Ovarian (OVCAR-3)	Colon **(**HT-29)
9a	Ph	H	15.75	11.40	28.12	16.33	18.18	9.88	19.54
9b	4-MeO-Ph	H	NT	0.49	>100	9.98	7.15	1.12	16.52
9c	4-NMe_2-Ph	H	NT	2.93	0.48	1.60	1.44	1.27	5.04
9d	3-NO_2-Ph	H	NT	40.07	0.62	16.11	>100	>100	>100
9e	2-Cl-Ph	H	NT	1.42	>100	6.10	3.85	0.01	10.81
9f	2-F-Ph	H	9.57	11.87	66.42	20.33	21.07	12.30	32.41
10a	Ph	Et	0.46	17.21	8.89	nt	>100	>100	>100
10b	4-MeO-Ph	Et	1.95	6.52	12.16	nt	8.56	6.52	9.62
10c	4-NMe_2-Ph	Et	63.33	54.87	2.60	6.61	58.95	2.42	65.65
10d	3-NO_2-Ph	Et	>100	>100	3.27	>100	>100	>100	>100
10e	2-Cl-Ph	Et	0.74	6.84	0.25	nt	4.12	3.97	88.87
10f	2-F-Ph	Et	>100	>100	>100	>100	>100	>100	>100

Table 1. *In vitro* antitumor activity (GI_{50} in µM)……..continued

11a	Ph	Ph	17.54	27.96	3.13	13.64	37.25	17.54	>100
11c	4-NMe_2-Ph	Ph	12.51	14.97	35.42	3.97	17.92	>100	38.06
11d	3-NO_2-Ph	Ph	1.59	2.36	0.19	1.53	3.91	0.83	2.28
11f	2-F-Ph	Ph	>100	>100	>100	>100	>100	>100	>100

NT = not tested. GI_{50}: concentration of compound necessary to promote 50% of growth inhibition after 48h of treatment, comparing to cells growing without treatment.

The *in vitro* antitumor activity of derivatives **18-23** are shown in **Table 2**. The changes in the nature of 4-thiazolidinone nucleus led to a decrease of activity for the derivatives of the *N*-(2-substituted-aryl-4-thiazolidinone)-*β*-carboline-3-carboxamide series compared to those from the *N'*-(2-ylidene-4-thiazolidinone)-*β*-carboline-3-carbohydrazides (**9-11**) series. However, a potent activity and high selectivity for glioma (U251) and ovarian (OVCAR-3) cancer cell lines was observed for some derivatives. Compound **18**, which contains a phenyl group in the 1-position of the *β*-carboline ring, as well as in the nitrogen atom of 4-thiazolidinone ring that and displayed GI_{50} values of 0.25 μM and 1.58 μM for glioma (U251) and ovarian (OVCAR-3) cell lines, respectively.

The compounds **20** and **22**, having the phenyl group at the 4-thiazolidinone moiety, and the 2-fluorophenyl and 3-nitrophenyl group at the *β*-carboline nucleus, showed potent activity and selectivity for ovarian (OVCAR) tumor cell lines, with GI_{50} values of 0.73 and 1.25 μM, respectively.

Furthermore, it was observed that the derivatives **22** and **23,** containing a substituent at the 2-position of the 4-thiazolidinone different of phenyl group, were no active against all tumor cell lines tested. This indicated the influence of nature of the substituent at 2-position of 4-thiazolidinone on antitumor activity.

Table 2. *In vitro* antitumor activity (GI_{50} in μM) of 1-(substituted-phenyl)-*β*-carboline 3-(*N*-2-substituted-aryl-4-thiazolidinone) derivatives **18-23**.

			Cell lines								
Comp	R^1	R^2	Glioma (U251)	Melanoma (UACC-62)	Breast (MCF-7)	Ovarian Resistant (NCI/ADR-RES)	Renal (786-0)	Lung (NCI-H460)	Prostate PC-3	Ovarian (OVCAR-3)	Colon **(**HT-29)
18	Ph	Ph	0.25	80.42	19.10	18.37	>100	>100	7.23	1.58	>100
19	4-NMe_2-Ph	Ph	64.49	nt	>100	64.49	>100	>100	nt	>100	>100
20	2-F-Ph	Ph	84.13	Nt	63.15	>100	>100	>100	nt	0.73	>100
21	Ph	*2*-thienyl	>100	>100	>100	>100	>100	>100	>100	>100	>100
22	3-NO_2-Ph	Ph	>100	Nt	>100	>100	>100	>100	nt	1.25	>100
23	Ph	2-Cl-Ph	>100	>100	>100	>100	>100	>100	>100	>100	>100

nt = not tested. GI_{50}: concentration of compound necessary to promote 50% of growth inhibition after 48h of treatment, comparing to cells growing without treatment.

2.2.2. *Cell death and cell cycle profile in 786-0 cell line for compound* ***9c***

In view of the potency of **9c**, this compound was chosen for further studies concerning cell death and cell cycle profile by flow cytometry. The studies were focused on the human renal adenocarcinoma cell line 786-0 since the derivative **9c** was able to inhibiting totally the growth and for killing 50% of this cell lines, at concentrations of 13 µM and 105 µM, respectively.

The exposure of phosphatidylserine (PS) on cell membrane surface signals for recognition and engulfment of dying cells, because cell membrane of viable cells exhibits substantial phospholipid asymmetry, with most of the PS residing on the inner leaflet of the plasma membrane [45]. One of the methods used for assessment of exposure of PS is the double staining for Annexin-V and 7-amino-actinomycin D (7-AAD) by flow cytometry analyses. Annexin-V binds to PS translocated to the outer face of the cell membrane during the initial process of apoptosis (early apoptosis). 7-AAD binds to the DNA of the cell and acts as an indicator of membrane structural integrity, since it is not able to enter viable cells and early apoptotic cells, thus indicating late apoptosis or necrosis. Double staining for annexin-V and 7-AAD without the annexin-V positive only stage can be a characteristic of necrosis or necroptosis (programmed necrosis). For cell death evaluation, we performed the Annexin-V/7-AAD double staining assay by flow cytometry.

Treatment with 25 µM of compound **9c** for 24 hours led to membrane integrity loss, since cells were double stained for annexin-V and 7-AAD or only stained for 7-AAD (**Figure 2A**). Cells treated with 25 µM of compound **9c** presented PS externalization, being 38.7 ± 0.3% of cells double stained for annexin-V and 7-AAD and 36.6 ± 2.3% of the cells stained with 7-AAD only (**Figure 2A**). To evaluate when cells were committed to cell death, we evaluated the influence of the treatment with compound **9c** for 18 and 15h. After 18h of treatment, 34.4 ± 1.5% of the cells were double stained for annexin-V and 7-AAD and 34.7 ± 0.2% of the cells stained with 7-AAD only (**Figure 2B**), meaning that there was no difference between 18 and 24h of treatments and that signalling for cell death may occur before 18 hours. In fact, after 15h of treatments, only 3.6 ± 0.8% of the cells were double stained for annexin-V and 7-AAD and 3.9 ± 0.1% of the cells stained with 7-AAD only, which was not different from the cells treated with vehicle, suggesting that compound **9c** at 25 µM induces cell death between 15 and 18 hours of treatment (**Figure 2C**). The fact that even in the

beginning there is no staining for annexin-V only suggest that the compound **9c** may induce necrotic or necroptotic cell death.

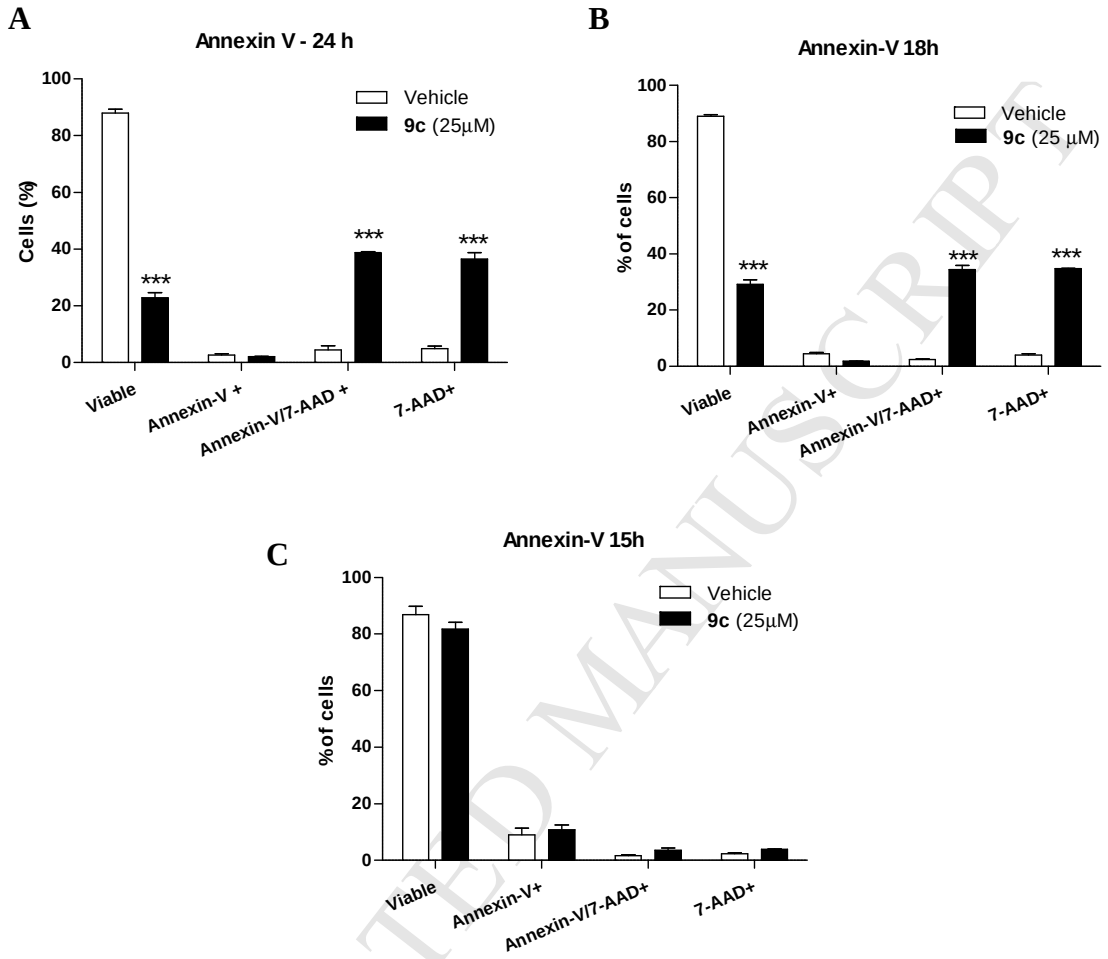


Figure 2. Compound **9c** (25 µM) induced cell death in 786-0 cells, with externalization of phosphatidylserine (Annexin-V positive cells – NEX-V+) and cell membrane degradation (7-AAD positive cells – 7AAD+). Cells were exposed to vehicle (DMSO) and Compound **9c** (25 µM) for 24 (A), 18 (B) and 15 hours (C) and analysed by flow cytometry, using Annexin V/7-AAD double staining assay. Results represented by mean ± standard error, in percentage of cells. Experiments were conducted three times, in triplicate.***$p<0.001$, Tukey's Multiple Comparison Test. ANOVA, statistically different from vehicle.

Alterations in the cell cycle profile are closely linked to cell death, either by being a consequence or preceding this phenomenon. Many of cancer cells treatment with anticancer agents usually result in cell cycle arrest, which subsequently leads the cells to enter apoptosis [44]. To evaluate 786-0 cell cycle profile after treatment with

compound **9c**, we performed cell cycle analysis by flow cytometry. For this study, cells were treated with 12.5 µM and 6.25 µM for 24 hours, non-cytotoxic concentrations. As a positive control, colchicine (1.25 nM) was employed, as it promoted cell cycle arrest on G2/M phase. The cell cycle distribution of treated 780-6 cells is presented in **Table 3**. As expected, colchicine (1.25 nM) increased the percentage of cells in G2/M phase (61.7 ± 2.1%), which was accompanied by a decreased percentage of cells in the G1 phase. No interference on the cell cycle profile was observed with compound **9c** at the concentration of 6.25 µM, but the treatment with 12.5 µM promoted a sub-G1 arrest, which indicates DNA fragmentation, typical of cell death.

Treatments with compound **9c** induced cell death in the renal human tumor cell line 786-0 between 15 and 18 hours after treatment with 25 µM. These cells were stained with annexin-V and 7-AAD, meaning exposure of phosphatidylserine and cell membrane degradation respectively, which could suggest death through necrosis or necroptosis. Further experiments will confirm this hypothesis. Treatments with lower concentrations (6.25 and 12.5 µM) did not affect the cell cycle profile of 786-0 cells.

Table 3. Cell cycle profile of 786-0 cells treated with vehicle (DMSO), colchicine (1.25 nM) and compound **9c** (6.25 and 12.5 µM) for 24 hours.

Treatment	***Sub-G1***	***G1***	***S***	***G2/M***
Vehicle	3.7 ± 0.9	47.1 ± 1.0	12.6 ± 0.7	36.5 ± 1.1
Colchicine (1.25 µM)	9.0 ± 0.6	18.3 ± 0.9***	11.0 ± 1.1	61.7 ± 2.1***
Compound **9c** (6.25µM)	2.3 ± 0.5	48.7 ± 0.8	11.9 ± 0.4	37.0 ± 1.7
Compound **9c** (12.5µM)	15.3 ± 5.0**	40.5 ± 2.9	12.4 ± 1.4	31.8 ± 6.5

Results represented by mean ± standard error, in percentage of cells. Experiments were conducted three times, in triplicate **p<0.01, ***p<0.001, Tukey's Multiple Comparison Test. ANOVA.

2.2.3. *Antiviral activity*

Some of the *β*-carboline-4-thiazolidinone derivatives synthesized were evaluated for their activity towards the *Herpes simplex* virus type-1(HSV-1). Compounds with EC_{50} > 100 µM were considered inactive. The cytotoxicity to Vero cells and the selectivity index for the active derivatives were also determined.

The antiviral assay results (**Table 4**) showed that regarding *N*'-(2-ylidene-4-thiazolidinone)-*β*-carboline-3-carbohydrazide series (**9-11**), the derivative **9f** with 2-

fluorophenyl group at 1-position was the most active with EC_{50} value of 19.57 μM. The introduction of the ethyl or phenyl groups at 3-position of the thiazolidinone ring of **9f** led to a loss of activity, resulting in the inactive derivatives **10f** and **11f**, respectively. Contrarily, the presence of ethyl group resulted in an important increment in the activity of derivative containing the 4-dimetylaminophenyl substituent at 1-position of the *β*-carboline. The EC_{50} values changed of 96.82 for **9c** to 6.78 μM for their N-ethyl analogue **10c**. Concerning to derivatives containing the phenyl group at the 3-position of the thiazolidinone ring **(11a, c, d, f)** only the compound **11d**, with the 3-nitrophenyl group attached to the 1-position of the *β*-carboline, showed significant antiviral activity with EC_{50} value of 17.24 μM.

From the comparison of EC_{50} data of derivatives **9-11** with those of *N*-(2-substituted-aryl-4-thiazolidinone)-*β*-carboline-3-carboxamides (**18-20** and **22, Table 4**), it was observed that nature of thiazolidinone moiety exerts significant influence in the antiviral potency. The N-(2-substituted-aryl-4-thiazolidinone)-carboxamide moiety in **18**, **19** and **22** confer a potent anti-HSV-1 activity for these derivatives, which presented EC_{50} values of 0.80, 2.15 and 2.02 μM, respectively. The derivative **20**, with 2-fluorophenyl group at 1-position, showed moderate activity with EC_{50} value of 30.28 μM. Based on the above information, it is possible to suggest that both groups appropriate in the 1-position and the 3-position of the *β*-carboline ring influence the antiviral activity.

Table 4. Antiviral activity against *Herpes simplex* virus type-1, cytotoxicity and selectivity index (SI) data for *β*-carboline-4-thiazolidinones **9-11** and **18-20,** and **22**

Compounds	R^1	R^2	**CC_{50}[a]± DP (μM)**	**EC_{50}[b]± DP (μM)**	**IS[c]**
9a	Ph	H	2493 ± 70.71	102.22 ± 1.732	24.39
9c	4-NMe_2-Ph	H	1744 ± 23.54	96.82 ± 2.000	18.02
9e	2-Cl-Ph	H	766 ± 6.35	>100	n d
9f	2-F-Ph	H	1073 ± 53.03	19.57 ± 1.170	54.88
10b	4-MeO-Ph	Et	1731 ± 5.77	26.57 ± 0.600	65.20
10c	4-NMe_2-Ph	Et	1649 ± 11.02	6.78 ± 0.517	243.40
10d	3-NO_2-Ph	Et	1792 ± 47.16	86.48 ± 1.040	20.73
10e	2-Cl-Ph	Et	1404 ± 88.0	>100	n d
10f	2-F-Ph	Et	2980 ± 17.68	>100	n d
11a	Ph	Ph	1478 ± 14.14	78.87 ± 1.607	18.75
11c	4-NMe_2-Ph	Ph	817 ± 14.14	49.98 ± 0.913	16.35
11d	3-NO_2-Ph	Ph	1168 ± 56.57	17.24 ± 0.985	67.78
11f	2-F-Ph	Ph	339 ± 17.68	>100	n d
18	Ph	Ph	506 ± 6.429	2.15 ± 1.732	235.0
19	4-NMe_2-Ph	Ph	467 ± 14.142	0.80 ± 2.000	592.5
20	2-F-Ph	Ph	637 ± 26.501	30.28 ± 1.628	21.0
22	3-NO_2-Ph	Ph	1080 ± 37.859	2.02 ± 0.494	533.9

[a] Concentration at which 50% cytotoxicity is observed. [b] Concentration at which 50% efficacy in antiviral assay is observed. [c] Selectivity index (CC_{50}/EC_{50}). [nd] not determined

3. Conclusions

In this work, novel hybrids *N'*-(2-ylidene-4-thiazolidinone)-*β*-carboline-3-carbohydrazides **(9-11)** and ***N***-(2-substituted-4-thiazolidinone)-*β*-carboline-3-carboxamides (**18-23**) have been synthesized and evaluated for their *in vitro* antitumor and anti-HSV-1 activities. The assay results showed that the nature of 4-thiazolidinone moiety and of the substituent attached at the 3- and 1- position of *β*-carboline nucleus, respectively, influenced the antitumor and antiviral activities.

Compound **11d,** containing the 2-nitrophenyl at C-1 of *β*-carboline nucleus, and the *N*-phenyl substituent at 4-thiazolidinone ring, was the most active for the *N'*-(2-ylidene-4-thiazolidinone)-*β*-carboline-3-carbohydrazides (**9-11**), showing GI_{50} values in the range of 0.83 to 3.81 μM for all cell lines tested. Compound **9c**, bearing the 4-dimethylaminophenyl group at C-1 of *β*-carboline, also presented high potency and induced cell death characterized by exposure of phosphatidylserine and cell membrane degradation in the 786-0 renal cancer cell line, with no interference in the cell cycle profile.

The changes in the nature of 4-thiazolidinone nucleus led to a decrease of activity for derivatives of the *N*-(2-substituted-aryl-4-thiazolidinone)-*β*-carboline-3-carboxamide series (**18-23**); however, a potent activity and high selectivity for glioma (U251) and ovarian (OVCAR) cancer cell lines was observed for some compounds of this series.

Furthermore, *β*-carboline-4-thiazolidinone hybrids showed potent antiviral activity against *Herpes simplex* virus type-1. The N-(2-substituted-aryl-4-thiazolidinone)-carboxamide moiety in **18**, **19** and **22** confer a potent anti-HSV-1 activity for these derivatives, which presented EC_{50} values of 0.80, 2.15 and 2.02 μM, respectively.

In summary, from our results it was possible to obtain new hybrids 4-thiazolidinone-*β*-carbolines with remarkable antitumor and antiviral activities, which can be considered as potential compounds for future studies in view to development of new anticancer and antiviral agents.

4. Experimental

4.1. General methods

All reagents were purchased from commercial suppliers. The reactions were monitored by thin layer chromatography conducted on Merk TLC plates (Silica Gel 60 F_{254}). ^{1}H and ^{13}C spectra were recorded in Varian spectrometer model Mercury plus BB 300 MHz and 75 MHz com deuterated solvents and TMS as internal standard, respectively, High Resolution Mass Spectra (HRMS) were recorded on a TOF Bruker Daltonics model IMPACT II spectrometer in a positive mode.

4.2. Synthesis

*4.2.1. General procedure for the synthesis of L-tryptophan methyl ester (**2**)*

To a suspension of 2.04 g (10 mmol) of commercial *L*-tryptophan (**1)** in 50 mL of MeOH was added dropwise concentrated sulfuric acid until complete solubilization. The mixture was kept under reflux, stirring for 48 hours and then cooled, neutralized with a solution of 5% sodium carbonate, and extracted with ethyl acetate (3 x 35 mL). The organic phase was dried with anhydrous sodium sulfate and after filtration, the solvent was removed in a rotary evaporator. The pure product was obtained in 95% yield.

*4.2.2. General procedure for the synthesis of cis + trans 1-(substituted-phenyl)-3-carbomethoxy-tetrahydro-β-carbolines (**3a-f**)*

To a solution of 1.09 g (5 mmol) of *L*-tryptophan methyl ester (**2)** in CH_2Cl_2 was added 5 mmol of the appropriate aldehyde and 2 equivalents of trifluoroacetic acid. The reaction mixture was kept at room temperature for 48 hours and after evaporation of the solvent and the remaining trifluoroacetic acid, the crude product was neutralized with a solution of 5% sodium carbonate and then extracted with ethyl acetate (3 x 35 mL). The organic phase was dried with anhydrous sodium sulfate and after filtration, the solvent was removed in a rotary evaporator. The solid obtained was washed thoroughly with methanol, providing a mixture of *cis* and *trans* 1-(substituted-phenyl)-3-carbomethoxy-tetrahydro-*β*-carboline.

*4.2.3. General procedure for synthesis of 1-(substituted-phenyl)-3-carbometoxy-β-carbolines (**4a-f**)*

A suspension of the corresponding *cis* and *trans* 1-(phenyl-substituted)-3-carbomethoxy-tetrahydro-*β*-carbolines **(3a-f)** (5 mmol) and sulfur (15 mmol) in xylene

(50 ml) was refluxed for 48 hours and subsequently at 0 °C for 3 hours. The precipitate formed was then filtered and washed with petroleum ether providing the *β*-carbolines.

*4.2.4. General procedure for synthesis of 1-(substituted-phenyl)-β-carboline-3-carbohydrazides (**5a-f**)*

To a suspension of 4.0 mmol of *β*-carbolines **(4a-f)** in ethanol (50 mL) were added 53 mmol of hydrazine hydrate 51%. This mixture was refluxed for 72 hours and at 0 °C for 3 hours to complete precipitation. The precipitate formed was filtered and washed with ethanol, which gave the corresponding pure products.

*4.2.5. General procedure for the synthesis of 1-(susbtituted-phenyl)-β-carboline-3-thiosemicarbazides (**6a-f**)*

To a suspension of 1-(substituted-phenyl)-*β*-carboline-3-carbohydrazide (1 mmol) **(5a-f)** in distilled water (30 ml) was added potassium thiocyanate (4 mmol) and drops of concentrated hydrochloric acid. The reaction was stirred and refluxed for a period of 48h. The precipitate formed was filtered on a sintered glass funnel and washed with distilled water.

*4.2.6. General procedure for the synthesis of 1-(substituted-phenyl)-β-carboline-3-thiosemicarbazides (**7a-f**) and (**8a, c-d, f**)*

To a solution of 1-(substituted-phenyl)-*β*-carboline-3-carbohydrazide **(5a-f)** (1 mmol) in ethanol (20 mL) were added ethyltioisocyanate and / or phenyl isothiocyanate (1 mmol). The reaction was stirred and refluxed for a period of 48h. The precipitate formed was filtered and washed with ethanol.

*4.2.7. General procedure for the synthesis of 1-(substituted-phenyl)-N'-(3-substituted-4-thiazolidinona-2-ylidene)-β-carbolina-3-carbohydrazides (**9a-f**), (**10a- f**) and (**11a, c-d, f**)*

To a solution of the *β*-carboline-3-thiosemicarbazides (**6a-f**), **(7a-f)** and **(8a, c-d, f)** (1.0 mmol) in ethanol (5 mL) was added ethyl bromoacetate (1.0 mmol), sodium acetate (4.0 mmol) and potassium hydroxide (1 0 mmol). The reaction mixture was stirred and refluxed for 48 hours. The precipitate formed was filtered and washed with ethanol.

*4.2.7.1. (Z)-1-(4-methoxyphenyl)-N'-(4-oxothiazolidin-2-ylidene)-9H-pyrido[3,4-b]indole-3-carbohydrazide **(9b)***. Yield: 62%; mp 256-257 °C. ^{1}H NMR (300 MHz,

DMSO-d_6): δ 3.90 (s, 3H, OCH_3), 4.05 (s, 2H, CH_2, H-5"), 7.21 (d, *J* = 8.7 Hz, 2H, H-3', H-5'), 7.32 (t, *J* = 7.3 Hz, 1H, H-6), 7.51 (t, *J* = 7.6 Hz, 1H, H-7), 7.71 (d, *J* = 7.3 Hz, 1H, H-8), 8.16 (d, *J* = 8.7 Hz, 2H, H-2', H-6'), 8.43 (d, *J* = 8.1 Hz, 1H, H-5), 8.83 (s, 1H, H-4), 10.66 (s, 1H, NH), 11.91 (s, 1H, NH), 11.99 (s, 1H, NH). ^{13}C NMR (75.45 MHz), DMSO-d_6): 34.6 (CH_2, C-5"), 55.4 ($O\underline{C}H_3$), 112.7 (CH, C-8), 112.9 (CH, C-4), 114.3 (2CH, C-3', C-5'), 120.3 (CH, C-6), 121.2 (C_0, C-4b), 122.1 (CH, C-5), 128.6 (C_0, C-4a), 129.7 (CH, C-7), 129.9 (C_0, C-1'), 130.1 (2CH, C-2', C-6'), 134.1 (C_0, C-9a), 138.5 (C_0, C-3), 140.4 (C_0, C-8a), 141.5 (C_0, C-1), 160.1 (C_0, C-4'); HRMS-ESI: calcd for $C_{22}H_{18}N_5O_3S$ $[M+H]^+$ 432,1130, found: 432.1099.

4.2.7.2. (Z)-1-(4-(dimethylamino)phenyl)-N'-(4-oxothiazolidin-2-ylidene)-9H-pyrido[3,4-b]indole-3-carbohydrazide ***(9c)***. Yield: 75%; mp > 270 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.10 (s, 6H, $N(Me)_2$), 3.97 (s, 2H, CH_2), 6.96 (d, *J* = 9.0 Hz, 2H, H-3', H-5'), 7.32 – 7.34 (m, 1H, H-6), 7.55 – 7.64 (m, 2H, H-7, H-8), 7.98 (d, *J* = 9.0 Hz, 2H, H-2', H-6'), 8.22, (d, *J* = 8.1 Hz, 1H, H-5), 8.76 (s, 1H, H-4). ^{13}C NMR (75.45 MHz), DMSO-d_6): 34.2 (CH_2, C-5"),, 39.8 ($2CH_3$), 112.1 (CH, C-4), 112.2 (2CH, C-3', C-5'), 112.7 (CH, C-8), 119.8 (C_0, C-4b), 120.6 (CH, C-6), 121.8 (CH, C-5), 128.3 (CH, C-7), 129.3 (C_0, C-4a), 129.4 (2CH, C-2', C-6'), 133.5 (C_0, C-1'), 133.8 (C_0, C-9a), 138.3 (C_0, C-1), 141.3 ($2C_0$, C-3, C-8a), 150.7 (C_0, C-4'), 150.8 (C=N, C-2"); HRMS-ESI: calcd for $C_{23}H_{20}N_6O_2S$ $[M+H]^+$ 445.1447, found 445.1162:

4.2.7.3. (Z)-1-(3-nitrophenyl)-N'-(4-oxothiazolidin-2-ylidene)-9H-pyrido[3,4-b]indole-3-carbohydrazide ***(9d)***. Yield: 81%; mp 212-214 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.04 (s, 2H, CH_2), 7.36 (t, *J* = 7.5 Hz, 1H, H-6), 7.65 (t, *J* = 7.5 Hz, 1H, H-7), 7.71 (d, *J* = 8.1 Hz, 1H, H-8), 7.95 (t, *J* = 7.8 Hz, 1H, H-5'), 8.42 (d, *J* = 7.8 Hz, 1H, H-6'), 8.49 (d, *J* = 7.8 Hz, 1H, H-5), 8.59 (s, 1H, H-2'), 8.64 (d, *J* = 7.8 Hz, 1H, H-4'), 9.00 (s, 1H, H-4), 10.80 (s, 1H, NH), 12.00 (s, 1H, NH), 12.10 (s, 1H, NH). ^{13}C NMR (75.45 MHz), DMSO-d_6): 34.6 (CH_2, C-5"), 112.7 (CH, C-8), 114.4 (CH, C-8), 120.6 (CH, C-6), 121.2 (C_0, C-4b), 122.3 (CH, C-5), 123.6 (CH, C-6'), 123.7 (CH, C-2'), 129.1 (CH, C-7), 130.4 (CH, C-5'), 130.5 (C_0, C-4a), 134.5 (C_0, C-1'), 135.3 (CH, C-4'), 138.3 (C_0, C-9a), 138.8 (C_0, C-3), 139.0 (C_0, C-8a), 141.7 (C_0, C-1), 148.3 (C_0, C-3'), 161.4 (C=N, C-2"), 172.1 (C=O, C-4"); HRMS-ESI: calcd for $C_{21}H_{15}N_6O_4S$ $[M+H]^+$ 447.0875, found: 447.0570.

*4.2.7.4. (Z)-1-(2-chlorophenyl)-N'-(4-oxothiazolidin-2-ylidene)-9H-pyrido[3,4-b]indole-3-carbohydrazide **(9e)***. Yield: 72%; mp 236-241 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.01 (s, 2H, CH_2), 7.34 (t, J = 7.3 Hz, H-6), 7.60 – 7.65 (m, 3H, H-3', H-4', H-5'), 7.72 – 7.79 (m, 2H, H-7, H-8, H-6'), 8.46 (d, J = 7.8 Hz, 1H, H-5), 8.95 (s, 1H, H-4), 10.46 (s, 1H, NH), 11.81 (s, 1H, NH), 11.93 (s, 2H, NH, NH). ^{13}C NMR (75.45 MHz), DMSO-d_6): 34.5 (CH_2, C-5"), 112.4 (CH, C-8), 114.1 (CH, C-4), 120.3 (CH, C-6), 121.0 (C_0, C-4b), 122.3 (CH, C-5), 128.9 (CH, C-7), 129.2 (CH, C-6'), 130.0 (C_0, C-4a), 130.0 (CH, C-4'), 130.7 (CH, C-5'), 132.1 (C_0, C-1'), 132.5 (CH, C-3'), 135.3 (C_0, C-2'), 138.2 (C_0, C-3), 139.7 (C_0, C-9a), 141.3 (C_0, C-1), 141.4 (C_0, C-8a), 161.2 (C=N, C-2"); HRMS-ESI: calcd for $C_{21}H_{15}ClN_5O_2S$ $[M+H]^+$ 436.0635, found: 436.0334.

*4.2.7.5. (Z)-1-(2-fluorophenyl)-N'-(4-oxothiazolidin-2-ylidene)-9H-pyrido[3,4-b]indole-3-carbohydrazide **(9f)***. Yield: 65%; mp > 250 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.52 (s, 2H, CH_2), 7.29 (td, J =7.1, 1.8 Hz, 1H, H-6), 7.44 – 7.50 (m, 2H, H-7, H-8), 7.54 – 7.68 (m, 3H, H-3', H-4', H-6'), 7.75 (t, J = 6.9 Hz, 1H, H-5'), 8.45. (d, J = 7.8 Hz, 1H, H-5), 8.88 (s, 1H, H-4), 11.09 (s, 1H, NH), 11.68 (s, 1H, NH). ^{13}C NMR (75.45 MHz), DMSO-d_6): 38.0 (CH_2, C-5"), 112.3 (CH, C-6'), 113.3 (CH, C-4), 116.4 (CH, C-8), 119.9 (CH, C-6), 120.9 (C_0, C-4b), 122.1 (CH, C-5), 124.9 (CH, C-7), 128.6 (C_0, C-4a), 129.1 (CH, C-4'), 131.0 (CH, C-3'), 131.8 (CH, C-5'), 135.0 (C_0, C-1), 136.4 (C_0, C-3), 140.4 (C_0, C-8a), 141.2 (C_0, C-9a), 157.1 (C_0, C-2'), 158.1 (C=N, C-2"), 167.3 (C=O, carbohydrazide), 182.4 (C=O, C-4"); HRMS-ESI calcd for $C_{21}H_{15}FN_5O_2S$ $[M+H]^+$ 420.0930, found: 420.0624.

*4.2.7.6. (Z)-N'-(3-ethyl-4-oxothiazolidin-2-ylidene)-1-phenyl-9H-pyrido[3,4-b]indole-3-carbohydrazide **(10a)***. Yield: 72%; mp 257-258 °C. ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 1,28 (t, J = 7.2 Hz, 3H, CH_3), 3.89-3.96 (m, 4H, CH_2, CH_2), 7.31 (t, J = 7.3 Hz, 1H, H-6), 7.48-7.54 (m, 2H, H-7, H-8), 7.57-7.61 (m, 3H, H-3', H-4', H-5'), 8.02 (d, J = 7.9 Hz, 2H, H-2', H-6'), 8.20 (d, J = 8,1 Hz, 1H, H-5), 8.84 (s, 1H, H-4). ^{13}C NMR (75.45 MHz, $CDCl_3$/CD_3OD): 12.1 ($CH_2\underline{C}H_3$), 33.1 (CH_2, C-5"), 38.5 ($\underline{C}H_2CH_3$), 112.2 (CH, C-8), 113.6 (CH, C-4), 120.6 (CH, C-6), 121.7 (CH, C-5), 128.2 (2CH, C-2', C-6'), 128.7 (CH, C-7), 128.9 (2CH, C-3', C-5'), 129.1 (CH, C-4'), 130.4 (C_0, C-1'), 137.7 (2C_0, C-1, C-9a), 138.0 (C_0, C-3), 141.4 (C_0, C-8a), 154.3 (C=N, C-2"), 162.1 (C=O, carbohydrazide), 170.8 (C=O, C-4"); HRMS-ESI: calcd for $C_{23}H_{20}N_5O_2S$ $[M+H]^+$ 430.1338, found: 430.1322.

4.2.7.7. (Z)-N'-(3-ethyl-4-oxothiazolidin-2-ylidene)-1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indole-3-carbohydrazide ***(10b)***. Yield: 63%; mp 158-160 °C. ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$): δ 1,32 (t, J = 7.2 Hz, 3H, CH_3), 3.93 – 4.00 (m, 4H, CH_2 CH_2), 3.93 (s, 3H, OCH_3), 7.16 (d, *J* = 9.0 Hz, 2H, H-3', H-5'), 7.35 (H-6), 7.56 – 7.64 (m, 2H, H-7, H-8), 8.02 (d, *J* = 8.7 Hz, 2H, H-2', H-6'), 8.22 (d, *J* = 8.1 Hz, 1H, H-5), 8.84 (s, 1H, H-4). ^{13}C NMR (75.45 MHz, $CDCl_3/CD_3OD$): 12.3 ($\underline{C}H_3CH_2$), 33.2 (CH_2, C-5"), 38.7 ($\underline{C}H_2CH_3$), 55.4 ($O\underline{C}H_3$), 112.2 (CH, C-8), 113.3 (CH, C-4), 114.4 (2CH, C-2', C-6'), 120.7 (CH, C-6), 121.9 (CH, C-5), 128.8 (CH, C-7), 129.6 (2CH, C-3', C-5'), 130.3 (C_0, C-1'), 130.4 (C_0, C-9a), 134.9 (C_0, C-1), 138.2 (C_0, C-3), 141.2 (C_0, C-8a), 154.2 (C=N, C-2"), 162.1 (C=O, carbohydrazide), 170.8 (C=O, C-4"); HRMS-ESI: calcd for $C_{24}H_{22}N_5O_3S$ $[M+H]^+$ 460.1443, found: 460.1430.

4.2.7.8. (Z)-N'-(3-ethyl-4-oxothiazolidin-2-ylidene)-1-(4-dimethylaminophenyl)-9H-pyrido[3,4-b]indole-3-carbohydrazide ***(10c)***. Yield: 73%; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$): δ 1.33 (t, *J* = 7.0 Hz, 3H, CH_3), 3.07 (s, 6H, $N(Me)_2$), 3.92 (s, 2H, CH_2), 3.98 (q, J = 7.0 Hz, 2H, CH_2), 6.94 (d, *J* = 9.0 Hz, 2H, H-3', H-5'), 7.36 (dt, *J* = 15.0, 7.0 Hz, 1H, H-6), 7.53 – 7.61 (m, 2H, H-7, H-8), 7.94 (d, *J* = 9.0 Hz, 1H, H-2', H-6'), 8.23 (d, *J* = 7.8 Hz, 1H, H-5), 8.85 (s, 1H, H-4). ^{13}C NMR (75.45 MHz, $CDCl_3/CD_3OD$): 12.2 ($\underline{C}H_3CH_2$), 29.6 (CH_2, C-5"), 38.6 ($\underline{C}H_2CH_3$), 40.3 (CH_3, $N(CH_3)_2$), 112.1 (CH, C-8), 112.5 (2CH, C-3', C-5'), 112.7 (CH, C-4), 120.6 (CH, C-6), 120.8 (CH, C-5), 122.0 (C_0, C-1'), 125.5 (C_0, C-4b), 128.5 (CH, C-7), 129.1 (2CH, C-2', C-6'), 130.0 (C_0, C-1), 134.9 (C_0, C-9a), 141.1 (C_0, C-3), 151.1(C=N, C-2").

4.2.7.9. (Z)-N'-(3-ethyl-4-oxothiazolidin-2-ylidene)-1-(3-nitrophenyl)-9H-pyrido[3,4-b] indole-3-carbohydrazide ***(10d)***. Yield: 71%; mp 284-286 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1,21 (t, *J* = 6.6 Hz, 3H, CH_3), 3.78 (q, J = 6.6 Hz, 2H, CH_2), 4.10 (s, 2H, CH_2), 7.34 (t, *J* = 7.0 Hz, 1H, H-6), 7.60 – 7.71 (m, 2H, H-7, H-8), 7.93 (t, J = 7.6 Hz, 1H, H-5'), 8.40 (d, *J* = 7.5 Hz, 1H, H-6'), 8.64 (d, *J* = 6.9 Hz, 1H, H-5), 8.92 (s, 1H, H-4), 8.96 (s, 1H, H-2'), 10.82 (s, 1H, NH), 12.13 (s, 1H, NH). ^{13}C NMR (75.45 MHz, DMSO-d_6): 12.2 ($\underline{C}H_3CH_2$), 32.7 (CH_2, C-5"), 37.6 ($\underline{C}H_2CH_3$), 112.7 (CH, C-8), 114.1 (CH, C-4), 120.5 (CH, C-6), 121.1 (CH, C-4'), 122.3 (CH, C-6'), 123.6 (CH, C-2'), 129.0 (CH, C-7), 130.3 (C_0, C-1'), 130.5 (CH, C-5'), 135.2 (CH, C-5), 138.1 (C_0, C-3), 138.8 (C_0, C-1), 141.1 (C_0, C-9a), 145.9 (C_0, C-8a), 148.3 (C_0, C-3'), 158.7 (C=N, C-2"), 160.0 (C=O, carbohydrazide), 171.1 (C=O, C-4"); HRMS-ESI: calcd for $C_{23}H_{19}N_6O_4S$ $[M+H]^+$ 475.1188, found: 475.1166.

4.2.7.10. (Z)-N'-(3-ethyl-4-oxothiazolidin-2-ylidene)-1-(2-chlorophenyl)-9H-pyrido[3,4-b]indole-3-carbohydrazide ***(10e)***. Yield: 82%; mp 174-176 °C. ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$): δ 1,31 (t, J = 7.05 Hz, 3H, CH_3), 3.92 – 3.99 (m, 4H, $2CH_2$), 7.48 – 7.53 (m, 2H, H-7, H-8), 7.58 – 7.72 (m, 4H, H-3', H-4', H-5', H-6'), 8.27 (d, J = 8.1 Hz, 1H, H-5), 8.94 (s, 1H, H-4), 8.95 (s, 1H, NH). ^{13}C NMR (75.45 MHz, $CDCl_3/CD_3OD$): 12.4 ($\underline{C}H_3CH_2$), 33.5 (CH_2, C-5"), 38.9 ($\underline{C}H_2CH_3$), 112.6 (CH, C-8), 114.6 (CH, C-4), 120.9 (CH, C-6), 122.0 (C_0, C-4b), 122.1 (CH, C-5), 127.5 (CH, C-7), 129.2 (CH, C-6'), 130.4 (C_0, C-4a), 130.7 (CH, C-4'), 131.8 (CH, C-3'), 133.7 (C_0, C-1'), 136.3 (C_0, C-2'), 136.5 (C_0, C-3), 137.9 (C_0, C-9a), 140.3 (C_0, C-1), 141.9 (C_0, C-8a), 155.5 (C=N, C-2"), 162.7 (C=O, carbohydrazide), 171.5 (C=O, C-4"); HRMS-ESI: calcd for $C_{23}H_{19}ClN_5O_2S$ $[M+H]^+$ 464.0948, found: 464.0896.

4.2.7.11. (Z)-N'-(3-ethyl-4-oxothiazolidin-2-ylidene)-1-(2-fluorophenyl)-9H-pyrido[3,4-b]indole-3-carbohydrazide ***(10f)***. Yield: 67%; mp 150-153 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1,33 (t, J = 7.0 Hz, 3H, CH_3), 3.95 (s, 2H, CH_2), 3.97 (q, J = 6.1 Hz, 2H, CH_2), 7.31 – 7.36 (m, 3H, H-6, H-3', H-6'), 7.41 (td, J = 7.6, 1.2 Hz, 1H, H-4'), 7.53 – 7.61 (m, 2H, H-7, H-8), 7.88 (td, J = 7.5, 1.8 Hz, 1H, H-5'), 8.26 (d, J = 7.8 Hz, 1H, H-5), 8.95 (s, 1H, H-4). ^{13}C NMR (75.45 MHz, DMSO-d_6): 12.3 ($\underline{C}H_3CH_2$), 33.2 (CH_2, C-5"), 38.7 ($\underline{C}H_2CH_3$), 112.6 (CH, C-8), 114.3 (CH, C-4), 116.3 (J = 21.6 Hz, CH, C-3'), 120.8 (CH, C-6), 121.6 (C_0, C-4b), 121.9 (CH, C-5), 124.9 (J = 3.3 Hz, CH, C-6'), 125.4 (J = 14.8 Hz, C_0, C-1'), 129.1 (CH, C-7), 130.3 (C_0, C-4a), 131.1 (J = 8.2 Hz, CH, C-4'), 131.8 (J = 3.9 Hz, CH, C-5'), 136.0 (C_0, C-1), 136.9 (C_0, C-3), 138.2 (C_0, C-8a), 141.3 (C_0, C-9a), 156.8 (J = 283.0 Hz, C_0, C-2'), 161.9 (C=N, C-2"), 162.1 (C=O, carbohydrazide), 171.0 (C=O, C-4"); HRMS-ESI: calcd for $C_{23}H_{19}FN_5O_2S$ $[M+H]^+$ 448.1243, found: 448.1153.

4.2.7.12. (Z)-N'-(4-oxo-3-phenylthiazolidin-2-ylidene)-1-phenyl-9H-pyrido[3,4-b]indole-3-carbohydrazide ***(11a)***. Yield: 65%; mp 283-285 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.27 (s, 2H, CH_2), 7.30 – 7.54 (m, 8H, H-3', H-4', H-5', H-2''', H-3''', H-4''', H-5''', H-6'''), 7.32 (t, J = 6.6 Hz, 1H, H-6), 7.57 – 7.61 (m, 1H, H-7), 7.65 – 7.69 (m, 1H, H-8), 8.19 (d, J = 6.3 Hz, 2H, H-2', H-6'), 8.44 (d, J = 7.2 Hz, 1H, H-5), 8.83 (s, 1H, H-4), 10.60 (s, 1H, NH), 12.90 (s, 1H, NH). ^{13}C NMR (75.45 MHz, DMSO-d_6): 33.1 (CH_2, C-5"), 112.7 (CH, C-8), 113.2 (CH, C-4), 120.3 (CH, C-6), 121.1 (C_0, C-4b), 122.2 (CH, C-5), 128.3 (2CH, C-3"', C-5'"), 128.6 (2CH, C-3', C-5'), 128.7 (CH, C-7), 128.9 (2CH, C-2'", C-6'"), 129.1 (2CH, C-2', C-6'), 130.0 (C_0, C-4a),

134.3 (C_0, C-1'), 134.9 (C_0, C-1'"), 137.3 (C_0, C-9a), 138.6 (C_0, C-3), 140.6 (C_0, C-1), 141.5 (C_0, C-8a), 158.5 (C=N, C-2"), 160.5 (C=O, carbohydrazide), 171.1 (C=O, C-4"); HRMS-ESI: calcd for $C_{27}H_{20}N_5O_2S$ $[M+H]^+$ 478.1338, found: 478.1115.

4.2.7.13. (Z)-1-(4-dimethylaminophenyl)-N'-(4-oxo-3-phenylthiazolidin-2-ylidene)-9H-pyrido[3,4-b]indole-3-carbohydrazide ***(11c)***. Yield: 68%; mp 278-279 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.04 (s, 6H, $N(Me)_2$), 4.28 (s, 2H, CH_2), 7.28 (t, J = 7.5 Hz, 1H, H-6), 6.96 (d, J = 8.7 Hz, 2H, H-3''', H-5'''), 7.41 (d, J = 7.5 Hz, 2H, H-3', H-5')7.47 – 7.50 (m, 1H, H-4'''), 7.54 (d, J = 7.5 Hz, 2H, H-2''', H-6'''), 7.47 – 7.70 (m, 2H, H-7, H-8), 8.05 (d, J = 8.7 Hz, 2H, H-2', H-6'), 8.38 (d, J = 7.8 Hz, 1H, H-5), 8.68 (s, 1H, H-4), 10.64 (s, 1H, NH), 11.82 (s, 1H, NH). ^{13}C NMR (75.45 MHz, DMSO-d_6): 33.2 (CH_2, C-5"), 111.8 (CH, C-4), 112.1 (2CH, C-3'", C-5'"), 112.7 (CH, C-8), 120.2 (CH, C-6), 121.2 (C_0, C-4b), 122.0 (CH, C-5), 124.7 (C_0, C-1'), 128.4 (2CH, C-3', C-5'), 128.7 (CH, C-7), 129.2 (2CH, C-2"', C-6'"), 129.4 (CH, C-4'"), 133.9 (C_0, C-1'"), 134.9 (C_0, C-9a), 138.3 (C_0, C-1), 141.4 ($2C_0$, C-8a, C-3), 150.9 (C_0, C-4'), 157.3 (C=N, C-2"), 160.5 (C=O, carbohydrazide), 171.1 (C=O, C-4"); HRMS-ESI: calcd for $C_{29}H_{25}N_6O_2S$ $[M+H]^+$ 521.1760, found: 521.1594.

4.2.7.14. (Z)-1-(3-nitrophenyl)-N'-(4-oxo-3-phenylthiazolidin-2-ylidene)-9H-pyrido[3,4-b]indole-3-carbohydrazide ***(11d)***. Yield: 75%; mp 256-258 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.26 (s, 2H, CH_2), 7.30 – 7.37 (m, 1H, H-6), 7.41 (d, J = 7.6 Hz, 2H, H-3''', H-5'''), 7.47 – 7.49 (m, 1H, H-4'''), 7.54 (d, J = 7.6 Hz, 2H, H-2''', H-6'''), 7.62 – 7.70 (m, 2H, H-7, H-8), 8.40 (d, J = 7.5 Hz, 1H, H-4'), 8.47 (d, J = 7.5 Hz, 1H, H-5), 8.63 (d, J = 7.8 Hz, 1H, H-6'), 8.87 (s, 1H, H-4), 8.96 (s, 1H, H-2'), 10.81 (s, 1H, NH), 12.13 (s, 1H, NH). ^{13}C NMR (75.45 MHz, DMSO-d_6): 33.0 (CH_2, C-5"), 112.6 (CH, C-8), 114.1 (CH, C-4), 120.1 (CH, C-6), 120.5 (C_0, C-4b), 122.3 (CH, C-5), 123.5 (CH, C-2'), 123.6 (CH, C-4'), 128.3 (2CH, C-3'", C-5'"), 128.7 (CH, C-7), 129.0 (CH, C-4'"), 129.1 (2CH, C-2'", C-6'"), 130.3 (CH, C-5'), 130.5 (C_0, C-1'), 134.4 (C_0, C-4a), 135.2 (C_0, C-1'"), 138.1 (C_0, C-1), 138.7 (C_0, C-9a), 139.0 (C_0, C-3), 141.6 (C_0, C-8a), 148.3 (C_0, C-3'), 159.7 (C=N, C-2"), 160.4 (C=O, carbohydrazide), 171.2 (C=O, C-4"); HRMS-ESI: calcd for $C_{27}H_{19}N_6O_4S$ $[M+H]^+$ 523.1188, found: 523.0923.

4.2.7.15. (Z)-1-(2-fluorophenyl)-N'-(4-oxo-3-phenylthiazolidin-2-ylidene)-9H-pyrido[3,4-b]indole-3-carbohydrazide ***(11f)***. Yield: 63%; mp 193-195 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.23 (s, 2H, CH_2), 7.31 (td, J = 7.2, 1.5 Hz, 1H, H-6), 7.40 (d,

J = 7.6 Hz, 2H, H-3’’’, H-5’’’), 7.45 – 7.50 (m, 3H, H-7, H-3’, H-6’), 7.53 (d, J = 7.5 Hz, 2H, H-2’’’, H-6’’’), 7.62 – 7.69 (m, 3H, H-8, H-5`, H-4’’’), 7.93 (td, J = 7.5, 1.5 Hz, 1H, H-4’), 8.45 (d, J = 7.8 Hz, 1H, H-5), 8.87 (s, 1H, H-4), 10.55 (s, 1H, NH), 11.85 (s, 1H, NH). ^{13}C NMR (75.45 MHz, DMSO-d_6): 33.1 (CH_2, C-5”), 112.4 (CH, C-8), 113.7 (CH, C-4), 116.3 (J = 209.0 Hz, CH, C-3’), 120.3 (CH, C-6), 120.8 (C_0, C-4b), 122.3 (CH, C-5), 124.9 (J = 3.15 Hz, CH, C-6’), 125.1 (C_0, C-4a), 128.3 (2CH, C-3’”, C-5’”), 128.7 (CH, C-7), 128.9 (CH, C-4’”), 129.1 (2CH, C-2’”, C-6’”), 131.3 (J = 8.4 Hz, CH, C-5’), 132.0 (J = 3.3 Hz, CH, C-4’), 133.2 (C_0, C-1’”), 134.8 (C_0, C-1’), 135.3 (C_0, C-9a), 136.4 (C_0, C-3), 138.6 (C_0, C-1), 141.3 (C_0, C-8a), 158.3 (C=O, carbohydrazide), 159.2 (J = 247.8 Hz, C_0, C-2’), 160.3 (C=N, C-2”), 171.1 (C=O, C-4”); HRMS-ESI: calcd for $C_{27}H_{19}FN_5O_2S$ $[M+H]^+$ 496.1243, found: 496.1029.

*4.2.8. General procedure for the synthesis of N’-arylidene-(1-substituted-phenyl)-β-carboline-3-carbohydrazides (**12-17**)*

To a solution of *β*-carboline-3-carbohydrazide **(5a, c, d, f)** (1.0 mmol) in DMF (3.0 mL) were added the appropriate aromatic aldehydes (1.2 mmol). The reaction mixture was irradiated in the domestic microwave oven at 60% power level for 1-3 min. The reaction was monitored by TLC. After all the starting material was consumed the reaction mixture was poured into water and the precipitate formed was collected by filtration and washed successively with water.

*4.2.9. General procedure for the synthesis of β-carboline-4-thiazolidinone (**18-23**)*

The emulsion of arylidene-*β*-carboline-carbohydrazide **(12-17)** (1.0 mmol) in toluene (10.0 mL) was added slowly mercapto acetic acid (2.0 mmol) and catalytic p-toluene sulfonic acid. The reaction medium was kept under stirring and reflux and TLC monitored the development of the reactions. After consumption of the starting material, the reaction was treated with a 5% solution of potassium carbonate until pH 8. Precipitate formed was collected by filtration on a sintered glass funnel and washed with water. For the derivatives, **19** and **23** no precipitate formation after treatment with base. In these cases, the reaction mixture was extracted with ethyl (3 x 20 mL). The organic phase was dried with anhydrous sodium sulfate, filtered and the solvent evaporated in vacuum.

*4.2.9.1. (Z)-N'-(4-oxothiazolidin-2-ylidene)-1-phenyl-9H-pyrido[3,4-b]indole-3-carbohydrazide **(9a)***. Yield: 70%; mp > 270 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.55 (s, 2H, CH_2, H-5"), 7.29 (t, J = 7.4 Hz, 1H, H-6), 7.52-7.72 (m, 5H, H-7, H-8, H-3', H-4', H-5'), 8.18 (d, J = 6.9 Hz, 2H, H-2', H-6'), 8.39 (d, J = 7.5 Hz, 1H, H-5), 8.80 (s, 1H, H-4), 10.20 (s, 1H, NH), 11.91 (s, 1H, NH), 11.99 (s, 1H, NH). ^{13}C NMR (75.45 MHz), DMSO-d_6): 34.6 (CH_2, C-5"), 112.6 (CH, C-8), 114.0 (CH, C-4), 120.9 (CH, C-6), 121.9 (C_0, C-4b), 122.0 (CH, C-5), 128.6 (2CH, C-2', C-6'), 129.1 (CH, C-7), 129.3 (2CH, C-3', C-5'), 129.5 (CH, C-4'), 130.8 (C_0, C-4a), 135.5 (C_0, C-1'), 137.9 (C_0, C-9a), 138.0 (C_0, C-3), 141.8 ($2C_0$, C-1, C-8a), 162.7 (C=N, C-2"); HRMS-ESI: calcd for $C_{21}H_{16}N_5O_2S$ $[M+H]^+$ 402.1025, found: 402.0736.

*4.2.9.2. N-(4-oxo-2-phenylthiazolidin-3-yl)-1-phenyl-9H-pyrido[3.4-b]indole-3-carboxamide **(18)***. Yield: 67%; mp 140-142 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.83 (dd, J = 15.0, 1.5 Hz, 1H, Hb), 3.99 (d, J = 15.0 Hz, 1H, Ha), 6.07 (s, 1H, CH), 7.35 – 746 (m, 6H, H-6, H-2''', H-3''', H-4''', H-5''', H-6'''), 7.49 – 7.64 (m, 6H, H-8, H-2', H-3', H-4', H-5', H-6'), 7.71 – 7.74 (m, 1H, H-7), 8.07 (d, J = 7.8 Hz, 1H, H-5), 8.27 (s, 1H, H-4), 9.22 (s, 1H, NH), 9.29 (s, 1H, NH); ^{13}C NMR 75.45 MHz, $CDCl_3$): δ 30.6 (CH_2, C-5"), 63.6 (CH, C-2"), 112.6 (CH, C-8), 113.8 (CH, C-4), 121.1 (CH, C-6), 121.9 (C_0, C-4b), 122.0 (CH, C-5), 128.1 (2CH, C-3'", C-5'"), 128.4 (2CH, C-3', C-5'), 129.0 (2CH, C-2', C-6'), 129.2 (2CH, C-2"', C-6"'), 129.3 (CH, C-4'"), 129.6 (CH, C-4'), 134.8 (C_0, C-4a), 137.2 ($2C_0$, C-1', C-1"'), 137.3 (C_0, C-9a), 137.5 (C_0, C-3), 140.6 (C_0, C-8a), 140.9 (C_0, C-1), 163.6 (C=O, carboxamide), 170.4 (C=O, C-4"); HRMS-ESI: calcd for $C_{27}H_{21}N_4O_2S$ $[M+H]^+$ 465.1385, found: 465.1133.

*4.2.9.3. N-(4-oxo-2-phenylthiazolidin-3-yl)-1-(4-dimethylaminophenyl)-9H-pyrido[3.4-b]indole-3-carboxamide **(19)***. Yield: 67%; mp 227-231°C ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.95 (s, 6H, $(CH_3)_2$), 3.78 (dd, J = 18.0, 1.8 Hz, 1H, Hb), 3.95 (dd, J = 15.0, 1.3, Hz, 1H, Ha), 6.01 (s, 1H, H-2''), 6.70 (d, J = 9.0 Hz, 2H, H-3'and H-5'), 7.30 – 735 (m, 1H, H-6), 7.34 (d, J = 9.0 Hz, 2H, H-2' and H-6') 7.48 – 7.62 (H-4'''), 7.49 – 7.54 (m, 1H, H-7), 7.51 (d, J = 7.5 Hz, 2H, H-3''' and H-5'''), 7.55 – 7.62 (m, 2H, H-2''' and H-6'''), 7.78 – 7.81 (m, 1H, H-8), 8.16 (d, J = 7.8, Hz, 1H, H-5), 8.65 (s, 1H, H-4); ^{13}C NMR 75.45 MHz, $CDCl_3$): δ 30.5 (CH_2, C-5"), 40.5 ($2CH_3$, $N(CH_3)_2$), 63.7 (CH, C-2"), 112.6 (3CH, C-8, C-3', C-5'), 114.3 (CH, C-4), 120.9 (CH, C-6), 121.8 (CH, C-5), 121.9 (C_0, C-4b), 123.5 (C_0, C-4a), 128.9 (2CH, C-3"', C-5'"), 129.0 (CH, C-4'"), 129.3 (CH, C-7), 129.5 (4CH, C-2', C-2"', C-6', C-6'"), 130.2 (C_0, C-1'"), 135.5 (C_0,

C-1'), 137.3 (C_0, C-9a), 137.7 (C_0, C-3), 141.6 (C_0, C-1), 151.6 (C_0, C-4'), 160.1 (C_0, C-8a), 164.8 (C=O, carboxamide), 170.5 (C=O, C-4"); HRMS-ESI: calcd for $C_{29}H_{26}N_5O_2S$ $[M+H]^+$ 508.1807, found: 508.1676.

4.2.9.4. N-(4-oxo-2-phenylthiazolidin-3-yl)-1-(2-fluorophenyl)-9H-pyrido[3.4-b]indole-3-carboxamide ***(20)***. Yield: 71%; mp 187-188 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.78 (dd, J = 15.0, 1.5 Hz, 1H, Hb), 3.92 (d, J = 15.9, Hz, 1H, Ha), 6.12 (s, 1H, H-2''), 7.33 – 7.38 (m, 5H, H-6, H-7, H-8, H-3''' and H-5'''), 7.43 – 7.50 (m, 1H, H-4'''), 7.49 (d, J = 7.8 Hz, 2H, H-2''' and H-6'''), 7.43 – 7.50 (m, 2H, H-6' and H-3'), 7.54 – 7.58 (m, 2H, H-4' and H-5'), 8.19 (d, J = 8.1, 1H, H-5), 8.84 (s, 1H, H-4); δ 30.4 (CH_2, C-5"), 63.2 (CH, C-2"), 112.0 (CH, C-8), 115.0 (CH, C-4), 121.3 (CH, C-6), 122.0 (C_0, C-4b), 122.3 (CH, C-5), 127.4 (CH, C-6'), 128.1 (2CH, C-3"', C-5'"), 129.1 (2CH, C-2"', C-6'"), 129.4 (CH, C-7), 129.6 (CH, C-5'), 130.0 (C_0, C-4a), 130.5 (CH, C-3'), 130.7 (CH, C-4'), 132.0 (CH, C-4"'), 133.0 (C_0, C-2'), 135.7 (C_0, C-1'), 135.9 (C_0, C-9a), 137.4 (C_0, C-1), 138.0 (C_0, C-3), 139.6 (C_0, C-1"'), 140.6 (C_0, C-8a), 163.8 (C=O, carboxamide), 170.0 (C=O, C-4"); HRMS-ESI: calcd for $C_{27}H_{20}FN_4O_2S$ $[M+H]^+$ 483.1291, found: 483.1013.

4.2.9.5. N-(4-oxo-2-thienylthiazolidin-3-yl)-1-phenyl-9H-pyrido[3.4-b]indole-3-carboxamide ***(21)***. Yield: 70%; mp 251-254 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.82 (d, J = 15.9 Hz, 1H, Hb), 3.94 (d, J = 15.9, Hz, 1H, Ha), 6.04 (s, 1H, H-2''), 7.30 – 7.38 (m, 5H, H-6, H-3', H-5', H-4'), 7.60 (d, J = 7.8 Hz, 2H, H-2' and H-6'), 7.58 – 7.63 (m, 1H, H-7), 7.74 (d, J = 8.4 Hz, 1H, H-8), 7.82 (d, J = 5.1 Hz, 1H, H-4'''), 8.12 (d, J = 4.0 Hz, 1H, H-3'''), 8.40 (d, J = 8.1 Hz, H-5), 8.75 (s, 1H, H-4); δ 29.5 (CH_2, C-5"), 62.2 (CH, C-2"), 112.9 (CH, C-8), 114.0 (CH, C-4), 120.7 (CH, C-6), 121.0 (C_0, C-4b), 122.1 (CH, C-5), 127.1 (CH, C-3'"), 127.6 (2CH, C-2', C-6'), 128.4 (CH, C-4'), 128.5 (2CH, C-3', C-5'), 128.9 (CH, C-5'"), 129.1 (CH, C-7), 130.1 (C_0, C-4a), 132.6 (CH, C-1'), 135.3 (C_0, C-8a), 137.3 (C_0, C-1'"), 138.1 (C_0, C-9a), 141.3 (C_0, C-3), 141.5 (C_0, C-1), 163.1 (C=O, carboxamide), 169.2 (C=O, C-4"); HRMS-ESI: calcd for $C_{25}H_{19}N_4O_2S_2$ $[M+H]^+$ 471.0949, found: 471.0701.

4.2.9.6. N-(4-oxo-2-phenylthiazolidin-3-yl)-1-(3-nitrophenyl)-9H-pyrido[3.4-b]indole-3-carboxamide ***(22)***. Yield: 83%; mp 172-174 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.89 (dd, J = 16.0, 1.5 Hz, 1H, Hb), 4.03 (dd, J = 16.2, 1.2 Hz, 1H, Ha), 6.15 (s, 1H, H-2''), 7.37 – 7.44 (m, 2H, H-7, H-8), 7.41 (d, J = 7.8 Hz, 2H, H-3''' and H-5'''), 7.47 (d, J =

8.4 Hz, 2H, H-2''' and H-6'''), 7.51 – 7.59 (m, 3H, H-6, H-2' and H-4'''), 7.83 (dt, J = 8.1, 1,0 Hz, 1H, H-5'), 7.95 (d, J = 8.1 Hz, 1H, H-6'), 8.15 (dd, J = 8.1, 1.0 Hz, 1H, H-5), 9.51 (s, 1H, H-4); δ 30.4 (CH_2, C-5"), 63.6 (CH, C-2"), 112.8 (CH, C-8), 115.1 (CH, C-4), 121.2 (CH, C-6), 121.7 (C_0, C-4b), 121.9 (CH, C-5), 123.8 (CH, C-4'), 123.9 (CH, C-2'), 128.0 (CH, C-7), 128.3 (2CH, C-3'", C-5'"), 129.0 (CH, C-4'"), 129.2 (2CH, C-2'", C-6'"), 129.9 (CH, C-5'), 130.2 (C_0, C-4a), 134.8 (CH, C-6'), 134.9 (C_0, C-1'), 137.5 (C_0, C-3), 138.8 (C_0, C-9a), 139.3 (C_0, C-1'"), 139.7 (C_0, C-1), 141.9 (C_0, C-8a), 149.3 (C_0, C-3'), 164.4 (C=O, carboxamide), 170.7 (C=O, C-4"); HRMS-ESI: calcd for $C_{27}H_{20}N_5O_4S$ $[M+H]^+$ 510.1236, found: 510.0914.

*4.2.9.7. N-[4-oxo-2-(2-chlorophenyl)thiazolidin-3-yl]-1-phenyl-9H-pyrido[3.4-b]indole-3-carboxamide (**23**).* Yield: 78%; mp 272-275 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.83 (dd, J = 15.0, 1.0 Hz, 1H, Hb), 3.92 (d, J = 15.6 Hz, 1H, Ha), 6.57 (s, 1H, H-2''), 7.28 – 7.42 (m, 5H, H-6, H-8, H-4', H-4''', H-5'''), 7.51 – 7.54 (m, 2H, H-7, H-6'''), 7.58 (d, J = 7.5 Hz, 2H, H-2'and H-6'), 7.68 (dd, J = 7.6, 1.3 Hz, 1H, H-3'''), 7.86 (d, J = 7.5 Hz, 2H, H-3' and H-5'), 8.17 (d, J = 8.1 Hz, 1H, H-5), 8.72 (s, 1H, H-4); δ 30.0 (CH_2, C-5"), 60.2 (CH, C-2"), 112.7 (CH, C-8), 114.6 (CH, C-4), 121.0 (CH, C-6), 121.9 (CH, C-5), 122.0 (C_0, C-4b), 128.5 (CH, C-5'"), 128.7 (CH, C-3'"), 129.1 (3CH, C-2', C-6', C-6'"), 129.4 (CH, C-7), 130.4 (C_0, C-4a), 130.4 (CH, C-4'"), 130.5 (CH, C-4'), 133.9 (C_0, C-2"'), 135.6 (C_0, C-1'), 135.7 (C_0, C-9a), 137.4 (C_0, C-1'"), 137.8 (C_0, C-1), 141.8 (C_0, C-3), 142.0 (C_0, C-8a), 165.1 (C=O, carboxamide), 170.9 (C=O, C-4"); HRMS-ESI: calcd for $C_{27}H_{20}ClN_4O_2S$ $[M+H]^+$ 499.0995, found: 499.0672.

4.3. Biological activities

4.3.1. Anticancer activity

The antitumor activity of the β-carboline-4-thiazolidinones was evaluated *in vitro* against nine different human cancer cell lines: U251 (glioma), UACC-62 (melanoma), MCF-7 (breast), NCI/ADR-RES (ovarian expressing multiple-drug-resistance phenotype), 786-0 (renal), NCI-H460 (non-small cell lung cancer), PC-3 (prostate), OVCAR-3 (ovarian) and HT-29 (colon) [46]. Stock and experimental cultures were grown in medium containing 5 mL RPMI 1640 (GIBCO BRL) supplemented with 5% fetal bovine serum (GIBCO BRL). Penicilin:Streptomicin mixture (1000 U/mL:1000 µg/mL, 1mL/L RPMI) was added to the experimental cultures. Cells in 96-well plates (100 µL cells well^{-1}) were exposed to sample

concentrations in DMSO/RPMI (0.25, 2.5, 25, 250 µg mL^{-1}) in triplicate at 37 ^{o}C, 5% of CO_2 in air for 48 h. The final DMSO concentration did not affect cell viability. Doxorubicin (0.025 to 25 µg/mL) was used as positive control. Before (T_0 plate) and after the sample addition (T_1 plates), cells were fixed with 50% trichloroacetic acid, and cell proliferation was determined by spectrophotometric quantification (540 nm) of cellular protein using the sulforhodamine B assay. Doxorubicin was employed as the positive control. All compounds were tested in triplicate each concentration. The GI_{50} (concentration expressed in µM that inhibits 50% of cell growth or cytostatic effect) were determined through non-linear regression analysis using the concentration–response curve for each cell line in software ORIGIN 8.0® (OriginLab Corporation). Compounds with GI_{50} values > 100 µM were considered inactive. The TGI (cytostatic activity) and LC_{50} (cytotoxic activity) parameters refer to the drug concentration for total growth inhibition and for killing 50% of the cells, respectively.

4.3.2. Measurement of phosphatidylserine externalization and membrane integrity

Phosphatidylserine externalization and membrane integrity was analysed using Guava® Nexin Assay Kit (Guava Technologies, Hayward, CA) in accordance with manufacturer's instructions. 786-0 cells were inoculated in 12 wells plate (3 x 10^4 cells/mL) and incubated for 24 hours at 37 ºC, 5% of CO_2 in air. Then, cells were treated with compound **9c** (25 µM) in DMSO/RPMI for 24 hours, harvested and resuspended at a density of 1 x 10^5 cells in 100 µL of supplemented medium. One hundred microliter of binding buffer containing annexin-V and 7-AAD were added on the cells and incubated in the dark for 20 min at room temperature. After, cells were analysed by flow cytometer (Guava Easycyte Mini-Guava Technologies, Hayward, CA). After, cells were analysed by flow cytometer (Guava Easycyte Mini-Guava Technologies, Hayward, CA). We collected 5.000 events, as suggested by the protocols of Guava Easycyte kits [47].

4.3.3. Cell cycle analyses

Cells cycle analyses were performed with the Guava®Cell Cycle reagent (Guava Technologies, Hayward, CA) in accordance with manufacturer's instructions. 780-0 cells were inoculated in 12 wells plate (3 x 10^4 cells/mL) and incubated for 24 hours at 37 ºC, 5% of CO_2 in air. Afterwards, cells were deprived of serum for 24 hours for cell cycle synchronization and then treated with compound **9c** (6.25 and 12.5 µM)

and colchicine (Sigma-Aldrich, 1.25 nM) in DMSO/RPMI, for 24 hours. After treatment, cells were harvested and resuspended at a density of 1 x 10^5 cells in 100 µL of PBS. The binding buffer containing propidium iodide (PI) was added to the cells (100 µL) and suspension was incubated in the dark for 20 minutes at room temperature. After, cells were analysed by flow cytometer (Guava Easycyte Mini-Guava Technologies, Hayward, CA). We collected 5.000 events, as suggested by the protocols of Guava Easycyte kits [48].

4.3.4. Statistical analyses

The results were expressed as the mean ± standard error and by analysis of variance (ANOVA), one-way followed by Tukey tests. P values lower than 0.05 ($p < 0.05$) were considered as indicative of significance and represented by: $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. The calculations were performed using the statistical software GraphPad Prism version 5.0, San Diego California, USA.

4.4. Antiviral activity

The compounds were evaluated for their cytotoxic potential in vitro according to the colorimetric technique of sulforhodamine B described by Skehan and *col* [54]: VERO cells were treated with various concentrations of compounds (1000 µg/mL, 500 µg/mL, 100 µg/mL, 10 µg/mL, 1 µg/mL and 0.1 µg/mL)in triplicate. It was used as control cells without the drug and acyclovir was used as a standard of HSV-1. Through the results of the antiviral activity assay was possible to analyze the effective concentration - EC_{50} is the concentration required to protect 50% of the cells against HSV-1; cytotoxic concentration - CC_{50} which is related to the toxic dose for 50% of the cells; and selectivity index - IS value which refers to how many times the analysis compound is more active to infection. So are active compounds that are experiencing IS > 1. The cytotoxicity was determined against cell line VERO - kidney African green monkey cells (ATCC CCL81). These cells were cultured in DMEM - Dulbecco's Modified Eagle Medium (Gibco®) supplemented with 10% fetal bovine serum (Gibco®) and gentamicin and maintained at 37ºC in an atmosphere of 5% CO_2. To obtain the viral suspensions VERO cell monolayers were cultured and infected with *Herpes simplex type I* KOS strain.

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Highlights

- Novel hybrids *β*-carboline-4-thiazolidinones were synthetized
- Compounds were evaluated for their *in vitro* antitumor and antiviral activities
- Compounds **9c** and **11d** displayed potent antitumor activity *in vitro*
- Compound **9c** induced cell death in the renal human tumor cell line 786-0
- Potent anti-HSV-1 activity was observed for derivatives **18**, **19** and **22**