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Synthesis and antitumoral activity of novel 3-(2-substituted-1,3,4-oxadiazol-5-yl) and 3-(5-substituted-1,2,4-triazol-3-yl) β-carboline derivatives

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ABSTRACT

Several novel 1-substituted-phenyl β-carbolines bearing the 2-substituted-1,3,4-oxadiazol-5-yl and 5-substituted-1,2,4-triazol-3-yl groups at C-3 were synthesized and evaluated for their in vitro anticancer activity. The assay results pointed thirteen compounds with growth inhibition effect (GI_{50} < 100 μM) for all eight different types of human cancer cell lines tested. The β-carbolines **7a** and **7h**, bearing the 3-(2-metylthio-1,3,4-oxadiazol-5-yl) group, displayed high selectivity and potent anticancer activity against ovarian cell line with GI_{50} values lying in the nanomolar concentration range (GI_{50} = 10 nM for both compounds). The 1-(*N*,*N*-dimethylaminophenyl)-3-(5-thioxo-1,2,4-triazol-3-yl) β-carboline (**8g**) was the most active compound, showing particular effectiveness on lung (GI_{50} = 0.06 μM), ovarian and renal cell lines. The potent anticancer activity presented for synthesized compounds **7a**, **7h**, and **8g**, together with their easiness of synthesis, makes these compounds promising anticancer agents.

1. Introduction

The β-carbolines alkaloids are a class of synthetic and naturally occurring compounds that possess a large spectrum of important pharmacological properties.[1–6] Recent reports[7–16] have pointed β-carboline alkaloids as potential antitumoral agents, which can act through multiple mechanisms, such as intercalating into DNA,[17] inhibiting Topoisomerase I and II,[18] CDK (cyclin-dependent kinases)[19,20] and IκK (IκB kinase complex).[21] Studies on a variety of synthetic β-carboline derivatives have demonstrated the influence of both molecule planarity and nature of the substituents in positions-1, -3 and -9 of the β-carboline skeleton on the antitumor activities.[7–16] Appropriate substituents on these positions could lead to more potent compounds with reduced toxicity. Consequently, the synthesis of novel β-carboline derivatives with different substituents are needed in order to provide more data for structure–activity relationship studies of this class of compounds.

Some studies demonstrated that β-carboline derivatives containing a phenyl-substituted group in 1-position present antitumoral activities.[4] Furthermore, several compounds having 1,3,4-oxadiazole and 1,2,4-triazole units were reported to possess antitumoral activities.[22–25] Therefore, in the present work we proposed the synthesis and in vitro anticancer activity evaluation of a series of novel 1-substituted-phenyl β-carbolines bearing the 2-substituted-1,3,4-oxadiazol-5-yl and 5-substituted-1,2,4-triazol-3-yl at C-3, expecting that the incorporation of these substituents may improve the antitumoral activities of β-carbolines.

2. Results and discussion

2.1. Chemistry

The synthetic routes for the preparation of 1,3-disubstituted β-carbolines are presented in Schemes 1 and 2. The methyl tetrahydro-β-carboline-3-carboxylates **3a**–**h** were prepared through a Pictet-Spengler condensation of the L-tryptophan **1** with appropriate aromatic aldehydes, in acid media, and subsequent esterification of the carboxylic acids **2a**–**h** with methanol and sulfuric acid. Conversion of the derivatives **3a**–**h** to the corresponding β-carboline-3-carbohydrazides **5a**–**h** was carried out by oxidation with sulfur in refluxing xylene of methyl 1,2,3,4-tetrahydro-9*H*-β-carboline-3-carboxilates **3a**–**h**, followed by reaction of methyl β-carboline-3-carboxylates **4a**–**h** with hydrazine hydrate, in ethanol under reflux, according to the procedures described in the literature.[12] For preparation of 3-(2-thioxo-1,3,4-oxadiazol-5-yl) β-carbolines **6a**–**h**, the key intermediates **5** were subjected to reaction with carbon disulfide in presence of KOH and ethanol, under reflux. The 1,3,4-oxadiazolyl β-carbolines **6a**–**h** were subsequently S-methylated with methyl iodide in the presence of K_2CO_3 at room temperature to afford the 3-(2-methylthio-1,3,

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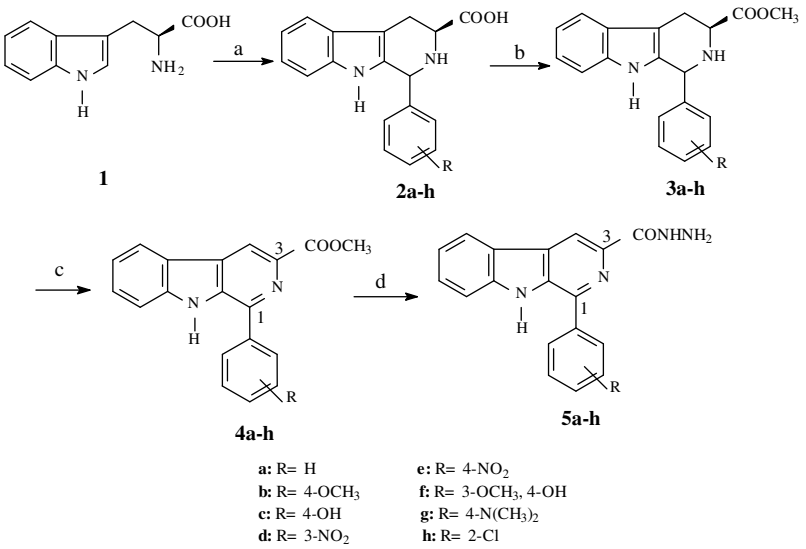
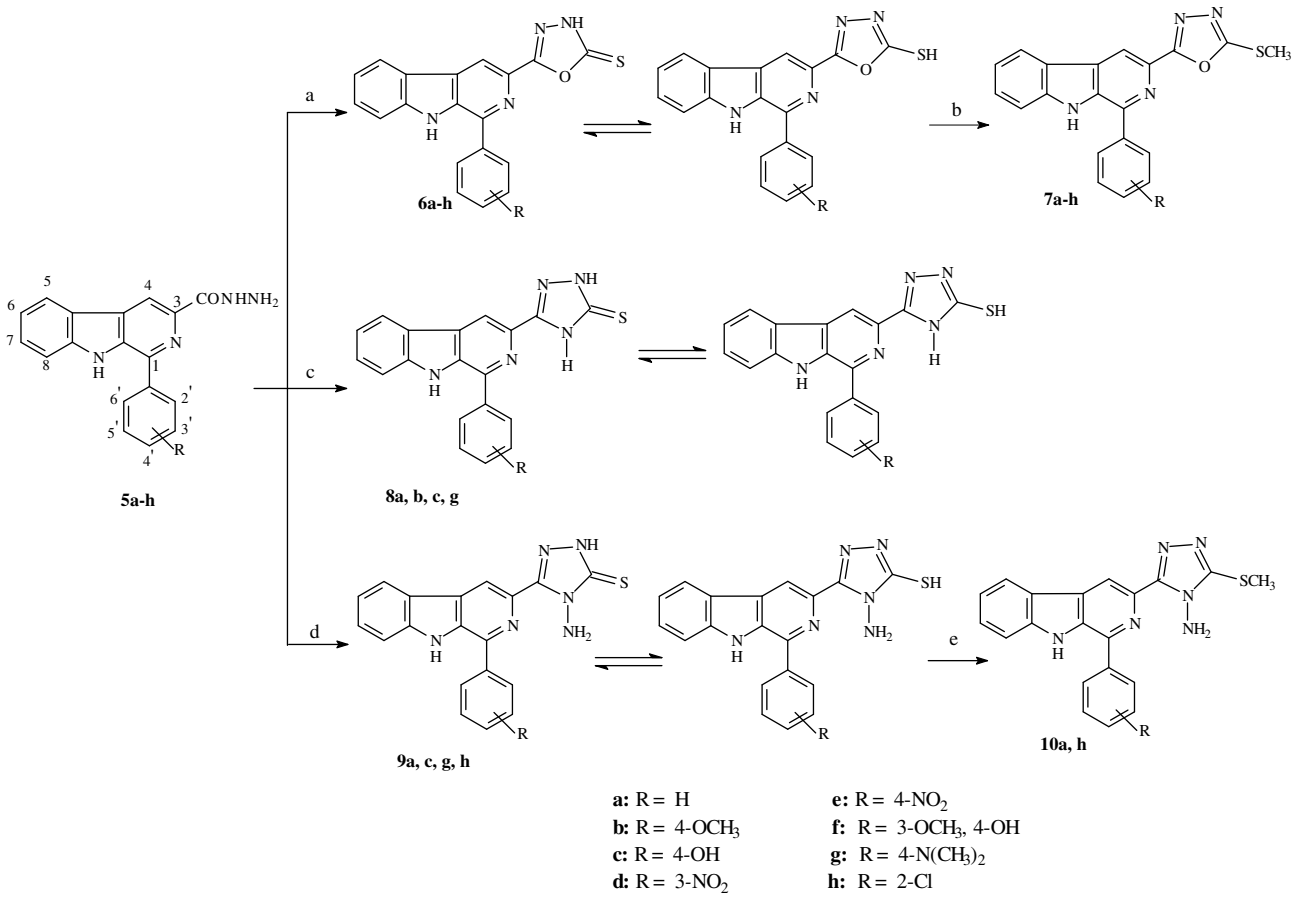


Scheme 1. Reagents and conditions: (a) acetic acid, RCHO, reflux, 2 h, and adjusted pH 5 with NH_4OH; (b) CH_3OH, H_2SO_4, reflux, 24 h; (c) S, xylene reflux 24 h, at 4 °C, 3 h; (d) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 48 h.



Scheme 2. Reagents and conditions: (a) CS_2/KOH, EtOH, reflux, 48 h, acidified dilute HCl; (b) CH_3I, K_2CO_3, THF, rt, 48 h; (c) KSCN, HCl, EtOH, reflux, 48 h, NaOH 2 M, reflux, 48 h; (d) CS_2/KOH, EtOH, reflux, 48 h, $NH_2NH_2.H_2O$, reflux, 4 days; (e) CH_3I, KOH, EtOH, rt, 48 h.

4-oxadiazol-5-yl) β-carbolines **7a–h**.[26] The 3-(5-thioxo-1,2,4-triazol-3-yl) β-carbolines **8a–c** and **8g** were prepared by reflux of corresponding compounds **5** with KSCN in ethanol and HCl and further alkaline cyclization of the thiosemicarbazides intermediates.[27] The synthesis of compounds **9a**, **9c**, and **9g–h** were accomplished by refluxing compounds **5** with carbon disulfide in presence of KOH, in ethanol, followed by reaction of the potassium dithiocarbazate salt with hydrazine hydrate, in ethanol, under reflux.[28] Reaction of **9a** and **9h** with methyl iodide and KOH produces the *S*-methyl derivatives **10a** and **10h**.[29]

The chemical structures of all novel compounds were confirmed by spectral data (^{1}H NMR, ^{13}C NMR, IR, and HR-ESIMS). The spectroscopic data are given in Section 4. The 3-(2-thioxo-1,3,4-oxadiazol-5-yl) β-carbolines **6** and the 3-(5-thioxo-1,2,4-triazol-3-yl) β-carbolines **8** and **9** may exist in thione-thiol tautomeric structures, as indicated by the –NH and –SH protons signals at 13.7–14.1 ppm in the ^{1}H NMR, as broad singlet integrating for one hydrogen each. In the state solid, these compounds are present in thione (–C=S) form, as indicated in their IR spectrum by absence of the absorption band at 2500 cm^{-1} (–SH stretching) and the presence of two absorption bands at 1230–1339 cm^{-1}, characteristic of the –C=S group for this class of compounds.

2.2. Anticancer activity

The anticancer activity of all synthesized 1,3-disubstituted β-carboline derivatives was evaluated in vitro against eight human tumor cell lines: melanoma (UACC-62), breast (MCF7), lung (NCI-460), leukemia (K-562), ovarian (OVCAR), prostate (PCO-3), colon (HT-29), and renal (786-0). Three response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each compound and cell line tested and the results were summarized in Tables 1 and 2. The GI_{50} values (growth inhibitory activity) (Table 1) refer to the drug concentration that produce a 50% reduction of cellular growth when compared to untreated control cells. The TGI (cytostatic activity) and

Table 1
GI_{50} values (in μM) and GI_{50} MG-MID (in μM) for compounds **6a–h**, **7a–h**, **8a–c**, **8g**, **9a**, **9c**, **9g–h**, **10a**, and **10h**

Compound	Melanoma UACC-62	Breast MCF-7	Lung NCI-460	Leukemia K-562	Ovarian OVCAR	Prostate PCO-3	Colon HT-29	Renal 786-0	MG-MID[a]
Cancer cell lines									
6a	>100	25.54	20.60	15.33	31.77	25.54	25.54	27.95	28.75
6b	16.76	24.88	9.20	26.44	>100	17.31	20.26	31.55	24.06
6c	>100	88.70	>100	21.47	>100	81.07	>100	94.88	78.65
6d	24.10	27.93	73.41	10.98	22.31	12.20	27.93	32.38	24.59
6e	37.99	27.56	>100	46.66	>100	26.01	31.34	73.13	48.20
6f	17.33	32.17	89.29	31.66	5.87	25.94	17.31	22.45	23.51
6g	23.82	18.84	19.09	9.55	14.72	14.72	40.46	14.72	17.90
6h	18.08	26.93	20.75	20.30	45.87	21.43	17.43	27.89	23.65
7a	99.48	12.19	3.42	0.43	0.01	23.62	16.22	32.04	4.65
7b	29.08	31.21	1.28	0.18	2.13	>100	>100	>100	12.05
7c	19.07	7.42	17.82	10.80	13.52	10.46	12.85	11.78	12.47
7d	16.38	79.75	24.47	21.32	21.32	44.18	68.14	>100	38.03
7e	>100	>100	>100	>100	>100	>100	>100	>100	100
7f	17.80	12.66	4.82	22.57	16.37	7.53	7.31	15.35	11.65
7g	>100	>100	>100	>100	>100	92.60	87.40	>100	97.39
7h	15.19	16.78	1.64	0.17	0.01	1.03	8.02	48.55	2.03
8a	6.36	>100	6.36	3.20	64.9	4.11	35.5	7.56	13.21
8b	66.8	>100	0.10	>100	>100	>100	>100	7.48	29.00
8c	10.6	19.8	6.69	>100	6.41	0.50	46.2	0.17	6.58
8g	1.74	8.63	0.06	11.1	1.09	1.37	8.63	0.04	1.23
9a	24.3	15.0	1.59	9.93	12.0	10.9	15.0	10.9	10.26
9c	22.5	61.2	0.15	29.9	27.5	25.5	34.0	0.26	8.87
9g	50.4	>100	0.20	>100	>100	>100	>100	0.97	23.65
9h	20.2	70.1	15.4	62.4	6.80	24.7	33.0	39.5	27.18
10a	>100	>100	>100	2.04	>100	>100	>100	>100	61.48
10h	18.6	20.1	13.9	1.92	11.2	22.2	20.4	16.8	13.07

[a] GI_{50} (in μM) mean-graph midpoint (MG-MID)=average sensitivity of all cell lines toward the test compounds.

Table 2
TGI (μM) and LC_{50} (μM; values in parentheses) and TGI and LC_{50} MG-MID (μM) for compounds **6a–h**, **7a–h**, **8a–c**, **8g**, **9a**, **9c**, **9g–h**, **10a**, and **10h**

	Melanoma UACC-62	Breast MCF-7	Lung NCI-460	Leukemia K-562	Ovarian OVCAR	Prostate PCO-3	Colon HT-29	Renal 786-0	MG-MID[a]
Cancer cell lines									
6a	>100	>100	>100	54.4 (>100)	>100	>100	>100	>100	92.67 (>100)
6b	47.2 (>100)	97.0 (>100)	>100	>100	>100	41.1 (>100)	55.3 (>100)	>100	75.36 (>100)
6c	>100	>100	>100	87.0 (>100)	>100	>100	>100	>100	98.27 (>100)
6d	>100	>100	>100	86.7 (>100)	>100	35.7 (>100)	>100	>100	86.36 (>100)
6e	>100	>100	>100	>100	>100	99.2 (>100)	>100	>100	99.90 (>100)
6f	47.9 (>100)	>100	>100	>100	>100	97.3 (>100)	>100	>100	90.90 (>100)
6h	80.1 (>100)	>100	>100	74.0 (>100)	>100	>100	>100	>100	93.67 (>100)
7a	>100	31.3 (>100)	74.7 (>100)	10.4 (74.7)	0.1 (1.6)	86.6 (>100)	>100	>100	26.03 (57.50)
7b	>100	>100	56.6 (>100)	>100	>100	>100	>100	>100	69.84 (>100)
7c	>100	23.4 (80.3)	73.4 (>100)	28.8(93.1)	35.0 (>100)	24.8 (59.2)	35.0 (>100)	24.8 (51.9)	37.28 (83.20)
7d	69.4 (>100)	>100	99.2 (>100)	81.5 (>100)	>100	>100	>100	>100	93.03 (>100)
7f	48.6 (>100)	28.1 (61.2)	>100	>100	59.8 (>100)	39.4 (>100)	39.4 (>100)	36.3 (>100)	68.06 (94.05)
7h	>100	51.9 (>100)	4.2 (>100)	68.4 (>100)	0.1(10.1)	3.8 (73.5)	99.6 (>100)	>100	16.56 (72.25)
8c	>100	>100	>100	>100	>100	40.7 (>100)	>100	23.8 (>100)	74.69 (>100)
8g	>100	37.0 (>100)	8.96 (>100)	>100	7.28 (47.9)	6.68 (>100)	51.5	1.24 (37.3)	17.85 (80.63)
9a	>100	32.5 (>100)	54.7 (>100)	>100	25.3 (52.5)	30.6 (>100)	86.9	49.4 (>100)	52.65 (92.26)
9c	>100	>100	12.2 (40.3)	>100	98.4 (>100)	>100	>100	94.1 (>100)	76.14 (89.26)
9g	>100	>100	71.8 (>100)	>100	>100	>100	>100	80.0 (>100)	93.30 (>100)
9h	86.1 (>100)	>100	38.0 (>100)	>100	49.9 (>100)	>100	>100	>100	79.73 (>100)
10h	59.0 (>100)	>100	41.8 (>100)	>100	38.4 (>100)	>100	>100	93.5 (>100)	73.86 (>100)

[a] TGI (μM) and LC_{50} (μM; values in parentheses) mean-graph midpoint (MG-MID) = average sensitivity of all cell lines toward the test compounds.

LC_{50} (cytotoxic activity) values parameters (Table 2) refer to the drug concentration for total growth inhibition and for killing 50% of the cells, respectively. Compounds with GI_{50} values < 100 μM were considered active. A mean-graph midpoint values (MG-MID) and selectivity of the compounds toward tumor cell lines were calculated for each of the parameters GI_{50}, TGI, and LC_{50}. MG-MID values furnished an averaged activity parameter for all cell lines. For the measurement of the selectivity, the MG-MID (μM) value for each compound was divided by their GI_{50}, TGI or LC_{50} values for a particular cell line. Selectivity (δ) was considered low if <10, moderate >10, and <100, and high if >100.

The anticancer assay results pointed thirteen compounds (**6d**, **6f**, **6g**, **6h**, **7a**, **7c**, **7f**, **7h**, **8g**, **9a**, **9c**, **9h**, and **10h**) with growth inhibition effect (GI_{50} < 100 μM) for all eight human cancer cell lines tested, showing GI_{50} (MG-MID) values in the range of 1.23–27.18 μM (Table 1). Compounds **7a**, **7h**, **8c**, **8g**, **9a**, and **9c** exhibited broad spectrum antitumor and potent activity at GI_{50} and TGI levels, showing effective growth inhibition GI_{50} (MG-MID) values of 4.65, 2.03, 6.58, 1.23, 10.26, and 8.87 μM (Table 1), respectively, and cytostatic activity TGI (MG-MID) values of 26.03, 16.56, 74.69, 17.85, 52.65, and 76.14 μM (Table 2), respectively. In addition, compounds **7a**, **7h**, **8g**, **9a**, and **9c** displayed cytotoxic efficacy with LC_{50} (MG-MID) values of 57.50, 72.25, 80.63, 92.26, and 89.26 μM (Table 2), respectively. Compounds **7a**, **7h**, and **8g** proved to be the most active derivatives in this study.

In relation to the sensitivity against some particular cell line, compounds **7a** and **7h** presented the highest growth inhibitory activity towards OVCAR ovarian cancer cell lines (GI_{50} = 0.01 μM; Table 1). Compound **8g** was particular effectiveness on NCI-460 lung (GI_{50} = 0.06 μM, TGI = 8.96 μM), OVCAR ovarian (GI_{50} = 1.09 μM, TGI = 7.28 and LC_{50} 47.9 μM) and 786-0 renal (GI_{50} = 0.04 μM, TGI = 1.24 and LC_{50} 37.3 μM) cell lines. Analysis of the selectivity data (Table 3) showed that compounds **7a**, **7h**, **8b**, and **9g** demonstrated high relative selectivity towards a particular tumor cell line (δGI_{50} in the range of 118.20–465.08; Table 3). Additionally, compounds **7a** and **7h** presented high selectivity at TGI level, with δTGI values 260.27 and 165.55, respectively.

From the series containing the 2-thioxo-1,3,4-oxadiazol-5-yl substituent at C-3 of the β-carboline system, compounds **6g** and **6h** with *p*-*N*,*N*-dimethylaminophenyl and *o*-chlorophenyl substituents at C-1, respectively, were the most actives with GI_{50} values minor of 50 μM against all human cell lines. In order to improve the activity we prepared the 2-methylthio-1,3,4-oxadiazol-5-yl derivatives **7a**–**h** by reaction of compounds **6** with methyl iodide and evaluated the effect of the S-methylation of **6a**–**h** on the antitumoral activity. The analysis of GI_{50} and TGI (MG-MID) values (Tables 1 and 2) showed an increase of the activities for compounds **7a**, **7b**, **7c**, **7f**, and **7h** compared to the 2-thioxo-1,3,4-oxadiazol-5-yl analogues. The β-carbolines **7a** and **7h**, with the phenyl and *o*-chlorophenyl substituents in position-1, respectively, were the most active derivatives for this series, displaying high selectivity and potent anticancer activity against OVCAR ovarian cell line both presenting GI_{50} of 0.01 μM and TGI of 0.1 μM (Tables 1 and 2). Also, both compounds displayed significant activity against leukemia (K-562) cell line with GI_{50} values of 0.43 and 0.17 μM, for **7a** and **7h**, respectively. A potent activity against this cell line was also observed for compound **7b** (GI_{50} = 0.18 μM), containing the *p*-methoxyphenyl group at position-1. The influence of the *S*-methyl group on activity was particularly significant for **6c**, with GI_{50} values increasing in the range of 21.47 to >100 μM (GI_{50} MG-MID = 78.65 μM) in **6c** to 7.42–19.07 μM (GI_{50} MG-MID = 12.47 μM) in the S-methylated analog **7c**. On the other hand, the S-methylation of the moderately active derivative **6e** (GI_{50} MG-MID = 48.20 μM) and of active **6g** (GI_{50} MG-MID = 17.90 μM) resulted in **7e** and **7g** (GI_{50} MG-MID values of 100 and 97.39 μM, respectively) with complete loss of the antitumoral activity.

The substitution of 2-thioxo-1,3,4-oxadiazol-5-yl group (compounds **6**) at C-3 of the β-carboline ring system for a 5-thioxo-1,2,4-triazol-3-yl group (compounds **8**) resulted in a increase of the antitumoral activity, particularly for **8c** and **8g** derivatives. The 1-(4-*N*,*N*-dimethylaminophenyl)-3-(5-thioxo-1,2,4-triazol-3-yl) β-carboline **8g** was the most active among all compounds tested, with particular effectiveness against NCI-470 lung, OVCAR ovarian, and 786-0 renal cell lines.

The 4-amination of the 1,2,4-triazol-3-yl heterocyclic ring of **8g** led to the compound **9g** with reduced activity, which showed only selective growth inhibition of the NCI-460 lung cell line with GI_{50} values of 0.20 μM. The 4-amino analog **9a** was de most active compound of this series, presenting GI_{50} values minor than 25 μM for all cell lines. S-methylation of **9a** and **9h** resulted in the reduction of the activity for the derivative **10a** (GI_{50} MG-MID = 61.48 μM; Table 1) and increase of the activity for **10h** (GI_{50} MG-MID = 13.07 μM; Table 1).

Analyzing the bioassays results we can observe an interestingly anticancer effect for 5-substituted-1,2,4-triazol-3-yl derivatives. Except for **10a**, the derivatives of this series showed a specific antitumoral activity towards human lung (NCI-460) and renal (786-0) cell lines with GI_{50} values in the range of 0.06–15.4 μM and 0.04–39.5 μM, respectively.

Table 3
Selectivity (δ) toward most sensitive tumor cell lines for compounds **7a**–**b**, **7h**, **8b**–**c**, **8g**, **9c**, **9g**, and **10a**

Compound	Cell lines	Selectivity[a]	
		(δ) for GI_{50}	(δ) for TGI
7a	K-562	10.9	
	OVCAR	465.08	260.27
7b	K-562	66.24	
7h	K-562	11.94	
	OVCAR	202.7	165.55
8b	NCI-460	289.96	
8c	PCO-3	13.17	
	786-0	38.77	
8g	NCI-460	20.46	
	786-0	30.83	14.39
9c	NCI-460	59.13	
	786-0	34.5	
9g	NCI-460	118.2	
	786-0	24.2	
10a	K-562	30.11	

[a] Selectivity was considered low if <10, moderate >10, and <100, high if >100.

3. Conclusions

In conclusion, several novel 3-(2-substituted-1,3,4-oxadiazol-5-yl) and 3-(5-substituted-1,2,4-triazol-3-yl) β-carboline derivatives displaying potential antitumoral activity were synthesized from the commercially available L-tryptophan in few steps and high yields. Particularly, compounds **7a**, **7h**, **8c**, **8g**, **9a**, and **9c** exhibited broad spectrum antitumor and potent activity at GI_{50}, TGI, and LC_{50} levels. Compounds **7a** and **7h** inhibits high and selectively OVCAR ovarian cell line with GI_{50} values lying in the nanomolar concentration range (GI_{50} = 10 nM). Compound **8g** was the most active among all compounds tested with GI_{50} values less than 11 μM against all tumor cell lines. The potent anticancer activity presented for the synthesized compounds, especially **7a**, **7h**, and **8g**, together with their easiness of synthesis makes these compounds promising anticancer agents. Our current investigation corroborated the previous reports that the antitumor activity of β-carboline derivatives is dependent of the substituents at the positions-1 and -3 of the β-carboline ring.

4. Experimental

4.1. Chemistry

4.1.1. General

Melting points were determined in a Micro-Química apparatus MQAPF-301 model and are uncorrected. ^{1}H and ^{13}C spectra were recorded in a Varian spectrometer model Mercury plus BB 300 MHz with DMSO as solvent and TMS as internal standard. HR-ESI mass spectra were recorded on a Q-Tof (Micromass) spectrometer in a positive ion mode. IR spectra were recorded as potassium bromide pellets on a BOMEM spectrometer model MB-100. All reagents were purchased from commercial suppliers.

4.1.2. General procedure for preparation of 1-(substituted phenyl)-3-(2-thioxo-1,3,4-oxadiazol-5-yl) β-carbolines (6a–h)

To a solution of derivatives **5a–h** (1 mmol) in ethanol (10 mL) was added carbon disulfide (5 mmol) and potassium hydroxide (1 mmol) at 0 °C. The resulting solution was refluxed for 48 h. The solvent was evaporated and the residue dissolved in water and acidified with a diluted solution of HCl. The solid obtained was filtered and crystallized from methanol.

4.1.2.1. 1-Phenyl-3-(2-thioxo-1,3,4-oxadiazol-5-yl) β-carboline (6a). Yield: 72%; mp 230.0–232.0 °C; IR (KBr) ν_{max}: 3056 (N–H), 1623 (C=N), 1556–1441 (C=C), 1368, 1237 (C=S), 1163 (C–O–C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.32 (t, J = 7.0 Hz, 1H, H-6), 7.55 (m, 1H, H-7), 7.57–7.69 (m, 4H, H-2′, H-3′, H-5′ and H-6′), 8.03 (d, J = 7.0 Hz, 2H, H-8 and H-4′), 8.44 (d, J = 7.2 Hz, 1H, H-5), 8.83 (s, 1H, H-4), 12.03 (s, 1H, 9-NH); ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 112, 113.9, 122.3, 120.5, 120.9, 128.6, 128.9, 129.0, 129.2, 129.5, 131.0, 134.0, 137.2, 141.6, 142.8, 161.2, 177.6; HR-ESIMS calcd for $C_{19}H_{12}N_4OS$ [M+H]$^+$: 345.0810; found 345.0681.

4.1.2.2. 1-(4-Methoxyphenyl)-3-(2-thioxo-1,3,4-oxadiazol-5-yl) β-carboline (6b). Yield: 75%; mp 180.0–182.0 °C; IR(KBr) ν_{max}: 3300 (N–H), 1610 (C=N), 1514–1432 (C=C), 1372, 1242 (C=S), 1178 (C–O–C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.88 (s, 3H, OMe), 7.20 (d, J = 8.7 Hz, 2H, H-3′, H-5′), 7.32 (t, J = 8.1 Hz, 1H, H-6), 7.60 (t, J = 8.1 Hz, 1H, H-7), 7.69 (d, J = 8.1 Hz, 1H, H-8), 8.02 (d, J = 8.7 Hz, 2H, H-2′, H-6′), 8.44 (d, J = 8.1 Hz, 1H, H-5), 8.80 (s, 1H, H-4), 11.9 (s, 1H, 9-NH); ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 55.2 (OMe), 112.4, 113.4, 114.4, 120.7, 121.3, 121.5, 128.9, 129.5, 129.6, 129.9, 130.7, 134.4, 141.4, 143.9, 160.4, 161.6, 178.3; HR-ESIMS: calcd for $C_{20}H_{14}N_4O_2S$ [M+H]$^+$ 375.0916; found 375.0750.

4.1.2.3. 1-(4-Hydroxyphenyl)-3-(2-thioxo-1,3,4-oxadiazol-5-yl) β-carboline (6c). Yield: 73%; mp 160.0–162.0 °C; IR(KBr) ν_{max}: 3349 (N–H), 1604 (C=N), 1557–1461 (C=C), 1366, 1266 (C=S), 1167 (C–O–C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 7.00 (d, J = 8.7 Hz, 2H, H-3′, H-5′), 7.34 (t, J = 8.1 Hz, 1H, H-6); 7.53 (t, J = 8.1 Hz, 1H, H-7), 7.66 (d, J = 8.1 Hz, 1H, H-8), 7.90 (d, J = 8.7 Hz, 2H, H-2′, H-6′), 8.24 (d, J = 8.1 Hz, 1H, H-5), 8.61 (s, 1H, H-4). ^{13}C NMR (75.45 MHz, $CDCl_3$/CD_3OD): δ 13.5 (C-4), 114.1(C-8), 116.7, 121.7, 122.6, 122.7, 129.8, 129.9, 130.9, 131.3, 132.4, 135.6, 143.1, 145.6, 160.0, 163.0, 180.2; HR-ESIMS: calcd for $C_{19}H_{12}N_4O_2S$ [M+H]$^+$ 361.0759; found 361.0707.

4.1.2.4. 1-(3-Nitrophenyl)-3-(2-thioxo-1,3,4-oxadiazol-5-yl) β-carboline (6d). Yield: 70%; mp 226.0–228.0 °C; IR(KBr) ν_{max}: 3079 (N–H), 1623 (C=N), 1525–1440 (C=C), 1349, 1235 (C=S), 1163 (C–O–C) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 7.34 (t, J = 7.8 Hz, 1H, H-6), 7.63 (d, J = 7.8 Hz, 2H, H-8, H-6′), 7.84 (t, J = 7.8 Hz, 1H, H-7), 8.27 (d, J = 7.8 Hz, 1H, H-4′), 8.36–8.42 (m, 2H, H-5, H-5′), 8.69 (s, 1H, H-4), 8.84 (d, J = 2,0 Hz, 1H, H-2′); ^{13}C NMR (75.45 MHz, $CDCl_3$/CD_3OD): δ 113.6, 115.2, 119.3, 122.0, 123.0, 124.6, 124.8, 130.5, 131.2, 132.1, 133.1, 135.9, 140.4, 141.2, 142.2, 143.4, 150.0, 162.9, 180.7; HR-ESIMS: calcd for $C_{19}H_{11}N_5O_3S$ [M+H]$^+$ 390.0549; found 390.0661.

4.1.2.5. 1-(4-Nitrophenyl)-3-(2-thioxo-1,3,4-oxadiazol-5-yl) β-carboline (6e). Yield: 72%; mp 260.0–262.0 °C; IR(KBr) ν_{max}: 3408 (N–H), 1623 (C=N), 1523–1448 (C=C), 1343, 1242 (C=S), 1175 (C–O–C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.35 (t, J = 7.8 Hz, 1H, H-6), 7.61–7.71 (m, 2H, H-7, H-8), 8.34 (d, J = 8.7 Hz, 2H, H-2′, H-6′), 8.46 (d, J = 7.8 Hz, 1H, H-5), 8.48 (d, J = 8.7 Hz, 2H, H-3′, H-5′), 8.93 (s, 1H, H-4), 12.14 (s, 1H, 9-NH); ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 112.8, 114.6, 120.0, 131.4, 122.5, 123.7, 124.0, 129.4, 129.9, 130.2, 134.1, 139.9, 141.7, 143.4, 147.5, 161.0, 178.1; HR-ESIMS: calcd for $C_{19}H_{11}N_5O_3S$ [M+H]$^+$ 390.0661; found 390.0549.

4.1.2.6. 1-(3-Methoxy-4-hydroxyphenyl)-3-(2-thioxo-1,3,4-oxadiazol-5-yl) β-carboline (6f). Yield: 68%; mp 188.0–190.0 °C; IR(KBr) ν_{max}: 3391 (N–H), 1645 (C=N), 1516–1435 (C=C), 1370, 1272 (C=S), 1173 (C–O–C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 4.01 (s, 3H, OMe), 7.05 (d, J = 8.1 Hz, 1H, H-5′), 7.36 (t, J = 7.8 Hz, 1H, H-6), 7.50 (dd, J = 8.1 Hz and 2.0 Hz, 1H, H-6′), 7.56 (d, J = 2.0 Hz, 1H, H-2′), 7.60–7.64 (m, 2H, H-7, H-8), 8.21 (d, J = 7.8 Hz, 1 H, H-5), 8.61 (s, 1H, H-4); ^{13}C NMR (75.45 MHz, $CDCl_3$/CD_3OD): δ 55.4; 112.8, 113.0, 113.5, 115.1, 120.7, 121.3, 121.5, 121.7, 128.9, 129.6, 130.5, 134.5, 141.5, 144.4, 147.4, 147.9, 161.4, 178.4; HR-ESIMS: calcd for $C_{20}H_{14}N_4O_3S$ [M+H]$^+$ 391.0865; found 391.0767.

4.1.2.7. 1-(4-*N*,*N*-Dimethylaminophenyl)-3-(2-thioxo-1,3,4-oxadiazol-5-yl) β-carboline (6g). Yield: 70%. mp: 186.0–188.0 °C; IR(KBr) ν_{max}: 3053 (N–H), 1607 (C=N), 1590–1454 (C=C), 1365, 1237 (C=S), 1167 (C–O–C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.05 (*s*, 6H, -N(Me)$_2$), 7.08 (d, J = 8.7 Hz, 2H, H-3′, H-5′), 7.31 (t, J = 8.1 Hz, 1H, H-6), 7.58 (t, J = 8.1 Hz, 1H, H-7), 7.70 (d, J = 8.1 Hz, 1H, H-8), 7.97 (d, J = 8.7 Hz, 2H, H-2′, H-6′), 8.43 (d, J = 8.1 Hz, 1H, H-5), 8.75 (s, 1H, H-4); ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 40.7, 112.0, 112.8, 113.0, 120.4, 120.9, 122.1, 128.7, 129.1, 129.5, 130.7, 133.4, 134.3, 141.3, 141.5, 143.2, 161.3, 177.5; HR-ESIMS: calcd for $C_{21}H_{17}N_5OS$ [M+H]$^+$ 388.1232; found 388.1078.

4.1.2.8. 1-(2-Chlorophenyl)-3-(2-thioxo-1,3,4-oxadiazol-5-yl) β-carboline (6h). Yield: 65%; mp 258.0–260.0 °C; IR(KBr) ν_{max}: 3185 (N–H), 1626 (C=N), 1595–1438 (C=C), 1315, 1247 (C=S), 1170 (C–O–C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.33 (m, 1H, H-6), 7.55–7.66 (m, 5H, H-7, H-8, H-4′, H-5′, H-6′), 7.72 (dd, J = 7.2 and 2.0 Hz, 1H, H-3′), 8.48 (d, J = 7.2 Hz, 1H, H-5), 8.94 (s, 1H, H-4), 11,8 (s, 1H, 9-NH); ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 112.6, 114.7, 120.5, 120.8, 122.5, 127.6, 128.7, 129.2, 129.8, 130.5, 130.9, 131.9, 132.5, 135.0, 136.0, 141.5, 142.0, 161.0, 177.6; HR-ESIMS: calcd for $C_{19}H_{11}ClN_4OS$ [M+H]$^+$ 379.0420; found 379.0175.

4.1.3. General procedure for the preparation of 1-(substituted-phenyl)-3-(2-methylthio-1,3,4-oxadiazol-5-yl) β-carbolines (7a–h)

To a solution of derivatives **6a**–**h** (0.6 mmol) in anhydrous THF (10 mL) was added potassium cabonate (0.6 mmol). The resulting solution was stirred at room temperature for 1 h. Methyl iodide (0.8 mmol) was then added and the mixture was stirred for an additional 48 h. The solvent was removed and the residue obtained was crystallized from methanol.

4.1.3.1. 1-Phenyl-3-(2-methylthio-1,3,4-oxadiazol-5-yl) β-carboline (7a). Yield: 53%; mp 216.0–218.0 °C; IR(KBr) ν_{max}: 3461 (N–H), 1625 (C=N), 1557–1447 (C=C), 1195 (C–O–C) 725 (C–S–C)

cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.80 (s, 3H, SMe), 7.34 (t, J = 7.2 Hz, 1H, H-6), 7.55–7.70 (m, 5H, H-7, H-8, H-3′, H-4′, H-5′), 8.04 (d, J = 8.1 Hz, 2H, H-2′, H-6′), 8.47 (d, J = 7.8 Hz, 1H, H-5), 8.97 (s, 1H, H-4), 11.85 (s, 1H, 9-NH); ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 14.4, 112.8, 114.1, 120.3, 120.9, 122.2, 128.5, 128.8, 129.1, 129.7, 131.5, 134.2, 137.3, 137.3, 142.7, 164.5, 165.9; HR-ESIMS: calcd for $C_{20}H_{14}N_4OS$ [M+H]$^+$ 359.0966; found 359.0862.

4.1.3.2. 1-(4-Methoxyphenyl)-3-(2-methylthio-1,3,4-oxadiazol-5-yl) β-carboline (7b). Yield: 50%; mp 235.0–237.0 °C; IR(KBr) ν_{max}: 3415 (N–H), 1624 (C=N), 1513–1447 (C=C), 1177 (C–O–C), 730 (C–S–C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.79 (s, 3H, SMe), 3.87 (s, 3H, OMe), 7.20 (d, J = 8.5 Hz, 2H, H-3′, H-5′), 7.32 (t, J = 7.5 Hz, 1H, H-6), 7.60 (t, J = 7.5 Hz, 1H, H-7), 7.69 (d, J = 7.8 Hz, 1H, H-8), 8.01 (d, J = 8.5 Hz, 2H, H-2′, H-6′), 8.43 (d, J = 7.8 Hz, 1H, H-5), 8.89 (s, 1H, H-4), 11.89 (s, 1H, 9-NH); ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 14.4, 55.4, 112.8, 113.5, 114.3, 120.3, 120.9, 122.2, 128.7, 129.4, 129.7, 130.1, 131.5, 133.8, 138.4, 141.7, 142.6, 160.1, 161.7; HR-ESIMS: calcd for $C_{21}H_{16}N_4O_2S$ [M+H]$^+$ 389.1072; found 389.1039.

4.1.3.3. 1-(4-Hydroxyphenyl)-3-(2-methylthio-1,3,4-oxadiazol-5-yl) β-carboline (7c). Yield: 45%; mp 252.0–254.0 °C; IR(KBr) ν_{max}: 3399 (N–H), 1622 (C=N), 1561–1447 (C=C), 1172 (C–O–C), 734 (C–S–C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.80 (s, 3H, SMe), 7.00 (d, J = 8.7 Hz, 2H, H-3′, H-5′), 7.30 (t, J = 7.8 Hz, 1H, H-6), 7.56 (t, J = 7.8 Hz, 1H, H-7), 7.67 (d, J = 7.8 Hz, 1H, H-8), 7.95 (d, J = 8.7 Hz, 2H, H-2′, H-6′), 8.40 (d, J = 7.8 Hz, 1H, H-5), 8.84 (s, 1H, H-4); ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 14.7, 113.4, 113.6, 118.9, 121.4, 122.3, 122.5, 129.5, 130.0, 130.5, 130.9, 132.4, 135.5, 142.9, 159.8, 167.8, 166.9; HR-ESIMS: calcd for $C_{20}H_{14}N_4O_2S$ [M+H]$^+$ 379.0420; found 379.0414.

4.1.3.4. 1-(3-Nitrophenyl)-3-(2-methylthio-1,3,4-oxadiazol-5-yl) β-carboline (7d). Yield: 47%; mp 220.0–223.0 °C; IR(KBr) ν_{max}: 3388 (N–H), 1623 (C=N), 1524–1437 (C=C), 1174 (C–O–C), 727 (C–S–C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.81 (s, 3H, SMe), 7.36 (t, J = 7.8 Hz, 1H, H-6), 7.63 (t, J = 7.8 Hz, 1H, H-7), 7.70 (d, J = 7.8 Hz, 1H, H-8), 7.95 (t, J = 8.1 Hz, 1H, H-5′), 8.42 (dd, J = 8.1 and 1.5 Hz, 1H, H-4′), 8.49 (d, J = 7.8 Hz, 1H, H-5), 8.54 (dd, J = 7.8 and 1.5 Hz, 1H, H-6′), 8.85 (brs, 1H, H-2′), 9.04 (s, 1H, H-4), 12.14 (s, 1H, 9-NH); ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 14.5, 112.9, 114.9, 120.5, 120.9, 122.4, 123.4, 123.6, 129.2, 130.3, 130.5, 131.6, 134.6, 135.1, 138.8, 140.1, 142.2, 148.2, 164.7, 165.6; HR-ESIMS: calcd for $C_{20}H_{13}N_5O_3S$ [M+H]$^+$ 404.0817; found 404.0804.

4.1.3.5. 1-(4-Nitrophenyl)-3-(2-methylthio-1,3,4-oxadiazol-5-yl) β-carboline (7e). Yield: 43%; mp 218.0–220.0 °C; IR(KBr) ν_{max}: 3337 (N–H), 1625 (C=N), 1574–1451 (C=C), 1173 (C–O–C), 728 (C–S–C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.81 (s, 3H, SMe), 7.41 (t, J = 7.5 Hz, 1H, H-6), 7.68 (t, J = 7.5 Hz, 1H, H-7), 7.70 (d, J = 7.5 Hz, 1H, H-8), 8.28 (d, J = 7.5 Hz,1H, H-5), 8.42 (d, J = 8.7 Hz, 2H, H-2′, H-6′), 8.82 (s, 1H, H-4), 9.34 (d, J = 8.7 Hz, 2H, H-3′, H-5′); ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 14.4, 112.0, 114.6, 116.8, 121.3, 122.4, 123.2, 126.6, 129.5, 130.0, 130.5, 131.4, 138.5, 139.9, 146.1, 156.3, 163.5, 166.2; HR-ESIMS: calcd for $C_{20}H_{13}N_5O_3S$ [M+H]$^+$ 404.0817; found 404.0804.

4.1.3.6. 1-(3-Methoxy-4-hydroxyphenyl)-3-(2-methylthio-1,3,4-oxadiazol-5-yl) β-carboline (7f). Yield: 40%; mp 210.0–212.0 °C; IR(KBr) ν_{max}: 3353 (N-H), 1625 (C=N), 1556–1448 (C=C), 1120 (C–O–C), 730 (C–S–C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2,80 (s, 3H, SMe), 3.89 (s, 3H, OMe), 7.00 (d, J = 8.1 Hz, 1H, H-5′), 7.36 (t, J = 7.8 Hz, 1H, H-6), 7.40 (dd, J = 8.1 Hz, J = 1.8 Hz, 1H, H-6′), 7.50 (d, J = 1.8 Hz, 1H, H-2′), 7.56–7.69 (m, 2H, H-7, H-8), 8.21 (d, J = 7.8 Hz, 1H, H-5), 8.84 (s, 1H, H-4); ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 14.4, 55.6, 112.5, 112.8, 113.3, 115.8, 120.5, 121.0, 121.6, 121.7, 128.7, 129.2, 131.5, 132.9, 133.8, 141.6, 143.4, 147.9, 148.3, 164.5, 165.9; HR-ESIMS: calcd for $C_{21}H_{16}N_4O_3S$ [M+H]$^+$ 405.1021; found 405.1204.

4.1.3.7. 1-(4-*N*,*N*-Dimethylaminophenyl)-3-(2-methylthio-1,3,4-oxadiazol-5-yl) β-carboline (7g). Yield: 48%; mp 168.0–170.0 °C; IR(KBr) ν_{max}: 3376 (N–H), 1624 (C=N), 1565–1435 (C=C), 1175 (C–O–C), 725 (C–S–C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.80 (s, 3H, SMe), 3.70 (s, 6H, N(Me)$_2$), 7.26 (t, J = 7.3 Hz, 1H, H-6), 7.56 (t, J = 7.3 Hz, 1H, H-7), 7.70 (d, J = 7.3 Hz, 1H, H-8), 8.19 (d, J = 8.7 Hz, 2H, H-3′, H-5′), 8.43 (d, J = 7.3 Hz, 1H, H-5), 8.52 (d, J = 8.7 Hz, 2H, H-2′, H-6′), 8.95 (s, 1H, H-4). ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 14.4, 114.2, 114.5, 119.4, 121.9, 122.5, 128.1, 129.7, 130.3, 131.2, 133.5, 139.7, 140.1, 146.9, 164.0, 166.2; HR-ESIMS: calcd for $C_{22}H_{19}N_5OS$ [M+H]$^+$ 402.1389; found 402.1459.

4.1.3.8. 1-(2-Chlorophenyl)-3-(2-methylthio-1,3,4-oxadiazol-5-yl) β-carboline (7h). Yield: 56%; mp 240.0–242.0 °C; IR(KBr) ν_{max}: 3453 (N–H), 1625 (C=N), 1562–1453 (C=C), 1198 (C–O–C), 730 (C–S–C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.78 (s, 3H, SMe), 7.33 (t, J = 7.0 Hz, 1H, H-6), 7.54–7.67 (m, 4H, H-7, H-4′, H-5′, H-6′), 7.59 (d, J = 8.0 Hz, 1H, H-8), 7.72 (dd, J = 7.2 and 1.5 Hz, 1H, H-3′), 8.46 (d, J = 7.8 Hz, 1H, H-5), 9.03 (s, 1H, H-4), 11.81 (s, 1H, 9-NH); ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 14.4, 112.5, 114.7, 120.3, 120.8, 122.5, 127.5, 128.8, 129.1, 129.8, 130.9, 131.2, 131.9, 132.5, 134.9, 136.1, 141.5, 164.6, 165.8; HR-ESIMS: calcd for $C_{20}H_{13}ClN_4OS$ [M+H]$^+$ 375.0916; found 375.0940.

4.1.4. General procedure for preparation of 1-(substituted-phenyl)-3-(5-thioxo-1,2,4-triazol-3-yl) β-carboline (8a–c, 8g)

A mixture of carbohydrazide (**5a**–**c**, **5g**) (1.0 mmol) and potassium thiocyanate (4.0 mmol) was refluxed for 48 h in ethanol (50 mL) containing 1.0 mL of concentrated hydrochloric acid. The precipitate formed was collected by filtration and dried. A solution of intermediate, which was used without further purification, in 2 N sodium hydroxide aqueous (25 mL) was refluxed for 48 h. After cooling to room temperature, concentrated hydrochloric acid was added. The precipitate was filtered and washed several times with distilled water and crystallized from methanol.

4.1.4.1. 1-Phenyl-3-(5-thioxo-1,2,4-triazol-3-yl) β-carboline (8a). Yield: 80%; mp 326.0–328.2 °C; IR(KBr) ν_{max}: 3419 (N–H), 1625 (C=N), 1576–1457 (C=C), 1321, 1237 (C=S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.30 (t, J = 7.5 Hz, 1H, H-6), 7.55–7.60 (m, 2H, H-7, H-4′), 7.62 (d, J = 7.5 Hz, 2H, H-3′, H-5′), 7.69 (d, J = 8.4 Hz, 1H, H-8), 8.32 (d, J = 7.5 Hz, 2H, H-2′, H-6′), 8.39 (d, J = 7.8 Hz, 1H, H-5), 8.76 (s, 1H, H-4),11.9 (s, 1H, 9-NH), 13.7 (s, 1H), 13.9 (s, 1H); ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 111.7, 112.8, 120.2, 120.9, 122.1, 128.7, 128.9, 129.0, 129.1, 130.2, 133.4, 133.9, 137.3, 141.6, 141.7, 151.6, 167.0; HR-ESIMS: calcd for $C_{19}H_{13}N_5S$ [M+H]$^+$ 344.0970; found 344.1064.

4.1.4.2. 1-(4-Methoxyphenyl)-3-(5-thioxo-1,2,4-triazol-3-yl) β-carboline (8b). Yield: 77%; mp 324.0–326.0 °C; IR(KBr) ν_{max}: 3418 (NH), 1625 (C=N), 1510–1425 (C=C), 1327, 1238 (C=S) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.88 (s, 3H, OMe), 7.19 (d, J = 8.7 Hz, 2H, H-3′, H-5′), 7.31 (t, J = 7.8 Hz, 1H, H-6), 7.59 (t, J = 7.8 Hz, 1H, H-7), 7.69 (d, J = 7.8 Hz, 1H, H-8), 8.04 (d, J = 8.7 Hz, 2H, H-2′, H-6′), 8.40 (d, J = 7.8 Hz, 1H, H-5), 8.85 (s, 1H, H-4), 11.0 (s, 1H, 9-NH), 13.7 (s, 1H), 13.8 (s, 1H). ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 55.4, 112.8, 114.2, 115.8, 120.4, 121.2, 122.0, 128.6, 129.3, 129.9, 130.1, 134.3, 137.2, 141.5, 141.6, 151.1, 160.0, 166.9; HR-ESIMS: calcd for $C_{20}H_{15}N_5OS$ [M+H]$^+$ 374.1075; found 374.1089.

4.1.4.3. 1-(4-Hydroxyphenyl)-3-(5-thioxo-1,2,4-triazol-3-yl) β-carboline (8c). Yield: 75%; mp 336.5–338.0 °C; IR(KBr) ν_{max}: 3303 (NH), 1625 (C=N), 1513–1434 (C=C), 1358, 1239 (C=S) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.03 (d, J = 8.7 Hz, 2H, H-3′, H-5′), 7.29 (t, J = 7.8 Hz, 1H, H-6), 7.58 (t, J = 7.8 Hz, 1H, H-7), 7.71 (d, J = 7.8 Hz, 1H, H-8), 8.18 (d, J = 8.7 Hz, 2H, H-2′, H-6′), 8.35 (d, J = 7.8 Hz, 1H, H-5), 8.70 (s, 1H, H-4), 11.9 (s, 1H, 9-NH), 13.7 (s, 1H), 13.8 (s, 1H). ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 111.0, 112.9, 115.5, 120.2, 121.0, 122.0, 127.7, 128.8, 130.0, 130.6, 133.0, 133.2, 141.8, 142.0, 151.4, 158.7, 166.9; HR-ESIMS: calcd for $C_{19}H_{13}N_5OS$ $[M+H]^+$ 360.0919; found 360.0906.

4.1.4.4. 1-(4-*N*,*N*-Dimethylaminophenyl)-3-(5-thioxo-1,2,4-triazol-3-yl) β-carboline (8g). Yield: 78%; mp 345.0–347.0 °C; IR(KBr) ν_{max}: 3449 (NH), 1625 (C=N), 1557–1411 (C=C), 1324, 1269 (C=S) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 3.10 (s, 6H, $N(Me)_2$), 7.28 (d, J = 8.4 Hz, 2H, H-3′, H-5′), 7.30 (d, J = 7.7 Hz, 1H, H-6), 7.58 (t, J = 7.7 Hz, 1H, H-7), 7.69 (d, J = 7.7 Hz, 1H, H-8), 8.30 (d, J = 8.4 Hz, 2H, H-2′, H-6′), 8.36 (d, J = 7.8 Hz, 1H, H-5), 8.70 (s, 1H, H-4), 11.9 (s, 1H, 9-NH), 13.7 (s, 1H), 13.9 (s, 1H). ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 41.9, 111.1, 112.8, 114.7, 120.2, 120.9, 122.0, 128.8, 130.0, 130.2, 133.0, 133.4, 141.5, 141.6, 141.8, 148.8, 151.4, 166.9; HR-ESIMS: calcd for $C_{21}H_{18}N_6O$ $[M+H]^+$ 387.1392; found 387.1368.

4.1.5. General procedure for preparation of 1-(substituted-phenyl)-3-(4-amino-5-thioxo-1,2,4-triazol-3-yl) β-carboline (9a, 9c, 9g–h)

To a cold solution (0–5 °C) of potassium hydroxide (1.5 mmol) and carbohydrazide **5** (1.0 mmol) in ethanol (25 mL) was added dropwise carbon disulfide (1.5 mmol) with stirring. The mixture was refluxed for 48 h and after cooled, diluted with 30 mL of ethyl ether. The precipitate formed was collected by filtration, washed with ethyl ether and dried. A suspension of the intermediate and hydrazine hydrate (20 mL) was refluxed for four days. The color of the reaction mixture changed to green, hydrogen sulfide was evolved and a homogeneous solution resulted. The reaction mixture was diluted with 20 mL of cold water and acidified with concentrated HCl. The product was collected by filtration, washed several times with cold water, dried and crystallized from ethanol.

4.1.5.1. 1-Phenyl-3-(4-amino-5-thioxo-1,2,4-triazol-3-yl) β-carboline (9a). Yield: 85%; mp. 358.0–360.0 °C; IR(KBr) ν_{max}: 3242, 3138 (NH), 1625 (C=N), 1557–1494 (C=C), 1348, 1239 (C=S) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 6.20 (brs, 2H, NH_2), 7.32 (t, J = 7.5 Hz, 1H, H-6), 7.54–7.61 (m, 2H, H-7, H-4′), 7.63 (d, J = 7.8 Hz, 2H, H-3′, H-5′), 7.72 (d, J = 8.1 Hz, 1H, H-8), 8.10 (d, J = 7.8 Hz, 2H, H-2′, H-6′), 8.38 (d, J = 7.8 Hz, 1H, H-5), 8.85 (s, 1H, H-4), 12.0 (s, 1H, 9-NH), 14,0 (s, 1H). ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 112.9, 114.1, 120.4, 120.9, 122.1, 128.7, 129.0, 129.1, 129.2, 129.2, 130.0, 133.3, 134.2, 137.2, 141.5, 141.8, 147.4, 163.9; HR-ESIMS: calcd for $C_{19}H_{14}N_6S$ $[M+H]^+$ 359.1079; found 359.1102.

4.1.5.2. 1-(4-Hydroxyphenyl)-3-(4-amino-5-thioxo-1,2,4-triazol-3-yl) β-carboline (9c). Yield: 80%; mp 355.0–356.8 °C; IR(KBr) ν_{max}: 3423 (NH), 1625 (C=N), 1557–1426 (C=C), 1352, 1281 (C=S) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.04 (d, J = 8.7 Hz, 2H, H-3′, H-5′), 7.32 (t, J = 7.5 Hz, 1H, H-6), 7.61 (t, J = 7.5 Hz, 1H, H-7), 7.71 (d, J = 7.5 Hz, 1H, H-8), 7.95 (d, J = 8.7 Hz, 2H, H-2′, H-6′), 8.37 (d, J = 7.5 Hz, 1H, H-5), 8.78 (s, 1H, H-4), 11.9 (s, 1H, 9-NH), 14.0 (s, 1H, NH). ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 112.9, 113.3, 115.9, 120.4, 120.9, 122.1, 127.5, 129.0, 129.8, 130.1, 132.9, 133.6, 141.8, 141.9, 147.1, 158.8, 164.0; HR-ESIMS: calcd for $C_{19}H_{14}N_6OS$ $[M+H]^+$ 375.1028; found 375.1168.

4.1.5.3. 1-(4-*N*,*N*-Dimethylaminophenyl)-3-(4-amino-5-thioxo-1,2,4-triazol-3-yl) β-carboline (9g). Yield: 82%; mp. 360.0–362.0 °C; IR(KBr) ν_{max}: 3411 (NH), 1624 (C=N), 1530–1426 (C=C), 1354, 1208 (C=S) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 3.07 (s, 6H, $N(Me)_2$), 7.13 (d, J = 8.4 Hz, 2H, H-3′, H-5′), 7.34 (t, J = 7.5 Hz, 1H, H-6), 7.63 (t, J = 7.5 Hz, 1H, H-7), 7.74 (d, J = 7.5 Hz, 1H, H-8), 8.01 (d, J = 8.4 Hz, 2H, H-2′, H-6′), 8.40 (d, J = 7.8 Hz, 1H, H-5), 8.82 (s, 1H, H-4), 12.1 (s, 1H, 9-NH), 14.1 (s, 1H). ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 40.8, 113.0, 113.4, 113.7, 120.7, 120.8, 122.3, 129.4, 129.9, 130.1, 132.3, 132.7, 141.2, 142.2, 146.5, 149.3, 150.2, 164.6; HR-ESIMS: calcd for $C_{21}H_{19}N_7S$ $[M+H]^+$ 402.1501; found 402.1694.

4.1.5.4. 1-(2-Chlorophenyl)-3-(4-amino-5-thioxo-1,2,4-triazol-3-yl) β-carboline (9h). Yield: 84%; mp 295.5–297.4 °C; IR(KBr) ν_{max}: 3245, 3137 (NH), 1626 (C=N), 1579–1434 (C=C), 1339, 1243 (C=S) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): 6.40 (s, 2H, NH_2), 7.33–7.80 (m, 7H, H-6, H-7, H-8, H-3′, H-4′, H-5′, H-6′), 8.39 (d, J = 7.8, 1H, H-5), 8.92 (s, 1H, H-4), 12.2 (s, 1H, 9-NH), 14.1 (s, 1H). ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 112.5, 114.8, 120.3, 120.7, 122.2, 127.6, 129.0, 129.1, 130.0, 130.8, 132.0, 132.4, 133.9, 134.2, 136.1, 140.5, 141.6, 147.3, 163.9; HR-ESIMS: calcd for $C_{19}H_{13}ClN_6S$ $[M+H]^+$ 393.0689; found 393.0782.

4.1.6. Procedure for preparation of 1-(phenyl)-3-(4-amino-5-methylthio-1,2,4-triazol-3-yl) β-carboline (10a,h)

To a suspension of **9a**,**h** (0.5 mmol) in ethanol (10 mL) was added dropwise, at 0°C an aqueous solution of potassium hydroxide (2 M, 1 mmol). To this mixture, methyl iodide (1 mmol) was added dropwise with vigorous stirring and the solution kept at room temperature for 48 h. Then, water (10 mL) was added, and the precipitate formed was separated by filtration, washed with water, and recrystallized from ethanol.

4.1.6.1. 1-Phenyl-3-(4-amino-5-methylthio-1,2,4-triazol-3-yl) β-carboline (10a). Yield: 50%; mp 228.0–230.0 °C; IR(KBr) ν_{max}: 3295 (NH), 1625 (C = N), 1594–1434 (C = C), 714 (C–S–C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.64 (s, 3H, SMe), 6.73 (s, 2H, NH_2), 7.31 (t, J = 7.8 Hz, 1H, H-6), 7.55–7.70 (m, 5H, H-7, H-8, H-3′, H-4′, H-5′), 8.10 (d, J = 7.5 Hz, 2H, H-2′, H-6′), 8.44 (d, 7.8 Hz, 1H, H-5), 8.88 (s, 1H, H-4), 11.8 (s, 1H, 9-NH). ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 13.2, 112.7, 112.8, 120.2, 120.9, 122.3, 128.5, 128.9, 129.2, 129.1, 130.3, 132.9, 136.5, 137.6, 141.2, 141.7, 151.8, 152.9; HR-ESIMS: calcd for $C_{20}H_{16}N_6S$ $[M+H]^+$ 373.1235; found 373.1394.

4.1.6.2. 1-(2-Chlorophenyl)-3-(4-amino-5-methylthio-1,2,4-triazol-3-yl) β-carboline (10h). Yield: 52%; mp 215.0–217.4 °C; IR(KBr) ν_{max}: 3200 (NH), 1625 (C=N), 1595–11436 (C=C), 716 (C–S–C) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.63 (s, 3H, SMe), 6.68 (s, 2H, NH_2), 7.30 -7.80 (m, 7H, H-6, H-7, H-8, H-3′, H-4′, H-5′, H-6), 8.45 (d, J = 7.8, 1H, H-5), 8.93 (s, 1H, H-4), 11.7 (s, 1H, 9-NH). ^{13}C NMR (75.45 MHz, DMSO-d_6): δ 13.3, 112.4, 113.5, 120.1, 120.8, 122.4, 127.7, 129.1, 129.6, 130.1, 130.7, 132.0, 132.3, 133.8, 135.8, 136.1, 139.9, 141.6, 151.6, 152.6; HR-ESIMS: calcd for $C_{20}H_{15}ClN_6S$ $[M+H]^+$ 407.0846; found 407.0858.

4.2. Anticancer assays

The synthesized compounds were evaluated in vitro against a eight-cell panel lines consisting of melanoma UACC-62, breast MCF-7, lung NCI-460, leukemia K-562, ovarian OVCAR, prostate PCO-3, colon HT29, and renal 786-0. The tests were performed by the colorimetric method with sulforrodamina B, according to NCI standard protocol and doxorubicin was used as positive control.[30] Assays were performed in a 96-well plate using four

concentrations at 10-fold dilutions (0.25 mg/mL to 250 mg/mL) for each test compound. The anticancer activity was deduced from dose–response curves and three dose–response parameters (GI_{50}, TGI, and LC_{50}) were calculated.

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