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Synthesis and antitumor activity of β-carboline 3-(substituted-carbohydrazide) derivatives

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ABSTRACT

A series of β-carboline derivatives bearing a substituted-carbohydrazide moiety at C-3 were synthesized and evaluated for their antitumor activity against eight human cancer cell lines. The β-carboline N-(substituted-benzylidene)carbohydrazides showed, in general, a greater antitumor activity than their *N*-(alkylidene)carbohydrazide analogues. The N^9-methylation of β-carboline N-(substituted-benzylidene) carbohydrazides resulted in a decrease of antitumor activity. Among compounds tested, the benzylidene-carbohydrazides **3**, **4**, **11**, **13**, **16**, **21** and **22** were the most active, possessing IC_{50} less than 10 μM for six of the eight tumor cell lines assayed. The derivative **4** displayed the most significant activity toward all tested cell lines, with a remarkable cytotoxicity against renal (786-0) cell lines (IC_{50} = 0.04 μM). Compound **4** was assayed for its in vivo antineoplastic activity in the Ehrlich solid carcinoma assay.

1. Introduction

Synthetic and naturally occurring compounds containing the β-carboline nucleus possess a large spectrum of important pharmacological properties, including potent antitumor activity. The potential of β-carboline compounds as anticancer agents have stimulated studies into their synthesis and structure–activity-relationship (SAR) with an aim to the improvement of their antitumor potential.

SAR studies on a variety of synthetic β-carboline derivatives have demonstrated that the introduction of appropriated substituents into position-1, -2, -3 and -9 of the β-carboline skeleton resulted in more potent antitumor derivatives, with reduced toxicity.[1–17] The anticancer mode of action of these alkaloids has been also widely investigated. Multiple mechanisms, such as DNA intercalation[4–17] and inhibition of Topoisomerases I and II,[18,19] IkB kinase (IKK),[20] cyclin-dependent kinases (CDKs),[21,22] mitogen activated protein kinase-activated protein kinase 2 (MK-2),[23] polo-like kinase (PLK1)[24] and kinesin-like protein Eg5[25] were pointed out from these investigations.

Our previous studies showed that β-carboline derivatives containing a phenyl-substituted group at C-1, and the 1,3,4-oxadiazole and 1,2,4-triazole units at C-3, presented significant antitumoral activities.[26,27] In order to evaluate the influence of different groups at 3-position and to provide additional data for structure–activity relationship studies of β-carbolines, we designed a series of new 1-phenyl-substituted β-carboline derivatives bearing a substituted-carbohydrazide moiety at C-3. Recent investigations of our research group showed that β-carboline-3-carbohydrazide derivatives were effective against poliovirus and herpes simplex virus (HSV-1).[28]

In continuation of our studies to develop novel potent antitumor agents, in this work we synthesized and evaluated the in vitro antitumor activity of a series of 1-(substituted-phenyl)-β-carbolines and 1-(substituted-phenyl)-9-methyl-β-carbolines, bearing a substituted-carbohydrazide moiety at C-3, against several human cancer cell lines. Also, the most active derivative was tested for its in vivo antineoplastic activity in the Ehrlich solid carcinoma assay.

2. Results and discussion

2.1. Chemistry

The synthetic strategies followed for the preparation of the β-carboline derivatives are depicted in Scheme 1. The methyl esters **1a–h** were prepared through a Pictet–Spengler condensation of the L-tryptophan with appropriate aromatic aldehydes, in acidic media, and subsequent esterification of the resulting carboxylic acids with methanol and sulfuric acid, as previously reported.[26,27] Reaction of **1a–h** with hydrazine hydrate afforded the respective carbohydrazides **2a–h**.[26,27] The N-(substituted benzylidene)-β-carboline-3-carbohydrazides (**3–26**) were synthesized according to a previously described methodology.[28] The compounds of the

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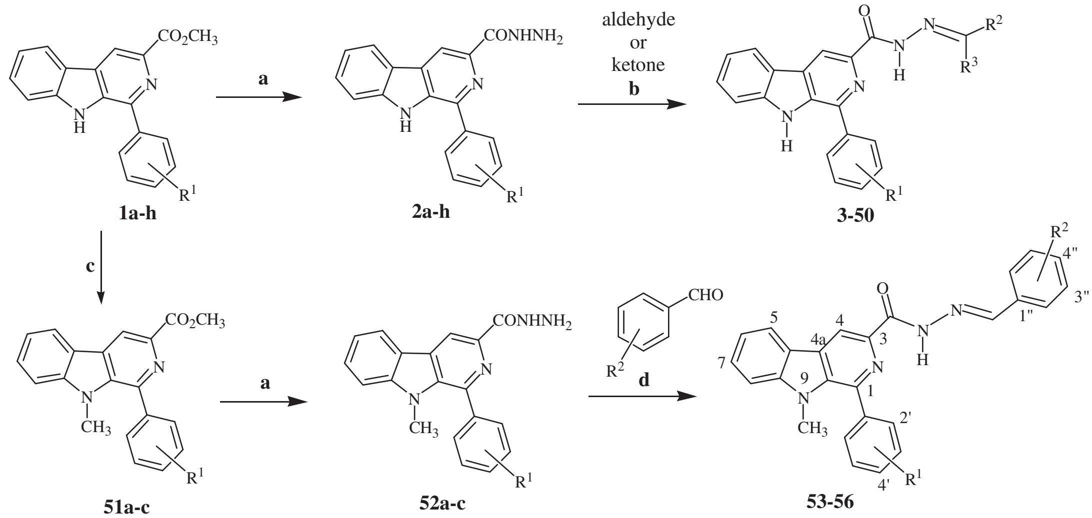

Scheme 1. Reagents and conditions: (a) $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 48 h; (b) aldehyde or ketone, EtOH, reflux 30 h; (c) CH_3I, NaOH, DMF, rt, 48 h; (d) EtOH, H_2SO_4 (cat), reflux 48 h.

N-alkylidene-carbohydrazide series **27–50** were prepared by the condensation of the **2a–h** with cyclohexanone, cyclopentanone and propanone, in ethanol under reflux (Scheme 1).

In order to evaluate the influence of a methyl group at the 9-position, some of the active carbohydrazides of the N-substituted-benzylidene series were N-methylated, using CH_3I and NaOH, in DMF at room temperature, to generate the corresponding derivatives **53–56** (Scheme 1).

All novel compounds were characterized by their spectral data (IR, EIMS, ^1H and ^{13}C NMR). Compounds **3–26** were characterized by comparison with NMR data previously reported.[23] The N-methylated derivatives **53–56** were characterized by the presence of an additional signal at δ_H/δ_C 3.50/33.5, corresponding to the N^9-methyl group attached to the β-carboline nucleus. The presence of an alkylidene-carbohydrazide moiety for the novel compounds **27–50** was confirmed by the signals at δ_C 154.8–162.8 (C=N) and δ_C 159.7–176.5 (C=O) in the ^{13}C NMR spectra, together with the signals for the respective alkyl groups.

2.2. Anticancer activity

2.2.1. In vitro assays

The in vitro anticancer activities of all synthesized compounds were evaluated against eight human cancer cell lines and the results are summarized in Table 1. Regarding the N-(substituted-benzylidene)-3-carbohydrazide series, the derivatives **3**, **4**, **11**, **13**, **16, 21** and **22** possesses potent antitumor activity with IC_{50} less than 10 μM against six of the eight human tumor cell lines assayed. Four compounds **(4**, **11**, **16** and **21)** were found to be the most potent derivatives of the *N*-benzylidene-carbohydrazide series, with IC_{50} values ranging of 0.04–10.38 μM against all the human tumor cell lines tested. Among these compounds, the derivative **4**, bearing a 3′-nitrophenyl group at C-1 and a 4-dimethylaminophenyl group attached to the carbohydrazide moiety, displayed the most significant activity toward all tested cell lines, with remarkable cytotoxicity against the renal (786-0) cell line, with an IC_{50} value of 0.04 μM. The derivatives **12** and **14** showed high selectivity toward ovarian cancer cell line (OVCAR) and were active against the herpes virus HSV-1 (EC_{50} = 22.9 μM) and poliovirus (EC_{50} = 2.67 μM), respectively, as demonstrated in our previous work.[28] On the other hand, the derivatives **9**, **10**, **24** and **25** were shown to be inactive against all cell lines, suggesting the importance of aromatic functionality with *m*-substituted electron withdrawal group for the activity. An analysis of the relation between the IC_{50} data and effect of the substituents at carboydrazide moiety showed that the phenyl and 2-chlorophenyl groups are present in four (compounds **11**, **13, 16** and **21)** of the seven more active compounds, demonstrating the importance of these substituents for the antitumor activity of the β-carboline *N*-benzylidene-carbohydrazide series.

In order to improve the antitumor activity, a series of β-carboline 3-(substituted-alkylidene)-carboydrazides (**27**–**50**) were synthesized and tested. Comparing the activity of the benzylidene and alkylidene groups in the carbohydrazide moiety indicated the decreased antitumor activity of derivatives possessing the later group. Among the compounds tested, only four derivatives presented significant cytotoxicity, with IC_{50} less than 20 μM against six of the eight human tumor cell lines, two of the cyclohexylidene (compounds **27** and **31**) and two of isopropylidene (compounds **42** and **44**) series. Concerning the activity against individual cell lines, the best activities were observed for compounds **31, 34** and **42** toward breast cell lines (MCF-7), with IC_{50} values ranging of 6.17–8.33 μM.

With the aim to evaluate the effect of a methyl group at the 9-position, some compounds of the alkylidene-carbohydrazide series (compounds **4**, **8**, **11** and **21)** were N^9-methylated, to afford the corresponding *N*-methyl derivatives **53**, **54**, **55** and **56**. The results of antitumor assays showed that the incorporation of the methyl group had a detrimental effect on the cytotoxicity. However, the N-methylation resulted in a remarkable increase in selectivity toward 786-0 renal cancer cells line for **53** and **54**, in addition to a small increase in their cytotoxic potential.

2.2.2. In vivo assays

In order to validate the in vitro antineoplastic activity of compounds, in vivo assays were used to assess the activity of the most active derivative **4**. The most active compound **4** was submitted to the Ehrlich solid carcinoma experimental model, which consists of a rapidly growing carcinoma inoculated into the mice paw, used to evaluate if the tested derivative will be able to reduce tumor development compared to a control group (vehicle treatment). This tumor model possesses a very aggressive behavior and is able to grow in almost all mice strains, which suggests that the recognition and immune responses to this tumor are independent of major histocompatibility complex. This characteristic suggests that the control of the Ehrlich tumor is related more with innate immunity, specially the inflammatory response, than with T cell responses.[29]

Table 1
In vitro antitumor activity (IC_{50} in μM) of 1-(substituted-phenyl) β-carboline 3-(substituted-carbohydrazide) derivatives **3–50** and **53–56**

Compd	R^1	R^2	R^3	Melanoma UACC-62	Breast MCF7	Lung NCI-460	Leukemia K-562	Ovarian OVCAR	Prostate PCO-3	Colon HT29	Renal 786-0
3	3-NO_2	C_6H_5	H	1.19	2.61	1.93	1.24	6.14	NT	1.93	3.76
4	3-NO_2	4-$N(CH_3)_2C_6H_5$	H	1.34	0.63	2.11	0.24	0.19	4.18	9.80	0.04
5	3-NO_2	4-$NO_2C_6H_5$	H	3.27	12.84	6.76	4.26	46.73	24.84	7.34	>100
6	3-NO_2	2-ClC_6H_5	H	35.71	14.12	28.89	68.08	>100	16.58	15.35	17.03
7	4-OCH_3	4-$OCH_3C_6H_5$	H	91.44	71.41	35.14	>100	>100	>100	>100	>100
8	4-OCH_3	C_6H_5	H	3.45	35.99	3.45	3.22	32.28	26.53	35.99	93.37
9	4-OCH_3	4-$N(CH_3)_2C_6H_5$	H	>100	>100	>100	>100	>100	>100	>100	>100
10	4-OCH_3	4-$NO_2C_6H_5$	H	>100	>100	>100	>100	>100	>100	>100	>100
11	4-OCH_3	2-ClC_6H_5	H	5.57	10.38	1.43	2.52	7.43	4.52	1.26	2.52
12	4-OH	4-$OCH_3C_6H_5$	H	3.85	>100	9.82	87.38	0.37	NT	95.59	61.98
13	4-OH	C_6H_5	H	3.18	8.94	19.83	4.31	1.26	2.25	4.32	4.32
14	4-OH	4-$N(CH_3)_2C_6H_5$	H	88.18	>100	69.54	>100	1.26	NT	>100	>100
15	4-OH	4-$NO_2C_6H_5$	H	4.46	12.27	>100	3.73	51.04	12.79	3.87	9.58
16	4-OH	2-ClC_6H_5	H	1.56	7.01	2.18	1.65	1.83	2.03	1.71	3.22
17	H	4-$OCH_3C_6H_5$	H	65.59	11.04	59.38	14.55	10.0	59.38	38.50	>100
18	H	C_6H_5	H	25.87	1568	8.49	79.54	10.56	11.63	>100	>100
19	H	4-$N(CH_3)_2C_6H_5$	H	87.14	75.91	>100	>100	75.91	87.14	>100	>100
20	H	4-$NO_2C_6H_5$	H	4.78	21.26	5.16	4.29	14.42	8.60	15.08	29.69
21	H	2-ClC_6H_5	H	2.16	6.13	4.57	1.45	2.84	2.52	1.25	2.16
22	4-NO_2	4-$OCH_3C_6H_5$	H	9.69	8.19	4.58	7.54	21.40	7.54	8.92	14.09
23	4-NO_2	C_6H_5	H	11.78	25.04	13.78	14.42	2.05	15.93	18.82	29.88
24	4-NO_2	4-$N(CH_3)_2C_6H_5$	H	>100	>100	>100	>100	>100	>100	>100	>100
25	4-NO_2	4-$NO_2C_6H_5$	H	>100	>100	>100	>100	>100	>100	>100	>100
26	4-NO_2	2-ClC_6H_5	H	2.53	18.02	38.08	6.35	4.55	18.03	3.85	2.75
27	4-OCH_3	$-CH_2(CH_2)_3CH_2-$		11.73	9.14	20.17	10.35	12.75	10.35	13.96	18.55
28	H	$-CH_2(CH_2)_3CH_2-$		31.17	32.50	68.87	55.90	78.05	27.51	47.30	>100
29	3-NO_2	$-CH_2(CH_2)_3CH_2-$		>100	>100	>100	87.14	>100	>100	>100	>100
31	4-OH	$-CH_2(CH_2)_3CH_2-$		7.86	8.33	13.20	10.27	13.20	7.68	10.27	14.96
32	4-$N(CH_3)_2$	$-CH_2(CH_2)_3CH_2-$		>100	>100	>100	NT	>100	>100	NT	>100
33	4-NO_2	$-CH_2(CH_2)_3CH_2-$		>100	25.64	>100	NT	>100	>100	>100	72.80
34	2-Cl	$-CH_2(CH_2)_3CH_2-$		21.64	6.19	54.14	NT	20.75	18.28	40.42	24.51
35	4-OCH_3	$-CH_2(CH_2)_2CH_2-$		76.37	>100	>100	>100	>100	>100	>100	>100
37	3-NO_2	$-CH_2(CH_2)_2CH_2-$		>100	>100	>100	>100	>100	>100	>100	>100
38	3-OH. 4-OCH_3	$-CH_2(CH_2)_2CH_2-$		>100	>100	>100	NT	>100	>100	NT	97.04
39	4-OH	$-CH_2(CH_2)_2CH_2-$		>100	78.14	>100	>100	>100	>100	>100	>100
40	4-$N(CH_3)_2$	$-CH_2(CH_2)_2CH_2-$		18.78	22.60	80.90	13.44	>100	20.41	55.58	>100
41	4-NO_2	$-CH_2(CH_2)_2CH_2-$		>100	>100	>100	>100	>100	>100	>100	>100
42	2-Cl	$-CH_2(CH_2)_2CH_2-$		11.67	7.37	13.78	NT	11.19	14.99	20.08	11.67
43	4-OCH_3	CH_3	CH_3	40.71	>100	>100	>100	>100	>100	>100	>100
44	H	CH_3	CH_3	10.13	14.17	19.77	6.69	26.37	15.39	19.77	16.06
45	3-NO_2	CH_3	CH_3	>100	>100	>100	>100	>100	>100	>100	>100
46	3-OH. 4-OCH_3	CH_3	CH_3	>100	>100	>100	>100	>100	>100	>100	70.00
47	4-OH	CH_3	CH_3	35.35	24.28	38.14	18.14	>100	19.70	NT	24.28
48	4-$N(CH_3)_2$	CH_3	CH_3	10.14	16.76	25.42	23.37	40.45	18.99	35.58	26.51
49	4-NO_2	CH_3	CH_3	>100	>100	>100	>100	>100	>100	>100	>100
50	2-Cl	CH_3	CH_3	19.13	26.72	>100	21.69	64.17	25.63	38.89	>100
53	3-NO_2	4-$N(CH_3)_2C_6H_5$	—	>100	>100	>100	NT	>100	>100	>100	1.24
54	4-OCH_3	C_6H_5	—	>100	>100	>100	>100	>100	>100	NT	1.39
55	4-OCH_3	2-ClC_6H_5	—	>100	14.04	>100	NT	>100	>100	>100	>100
56	H	2-ClC_6H_5	—	>100	>100	>100	NT	>100	>100	>100	>100

NT = not tested; Compounds with IC_{50} >100 μM were considered not active.

Inflammation, an environment rich in inflammatory cells, growth factors, activated stroma, and DNA damage, is a physiologic process directly involved in the Ehrlich tumor development. Several studies have established the link between cancer and chronic inflammatory processes. Epidemiological evidence supports such relationships and suggests that more than 25% of cancer diseases are related to chronic infections or other types of inflammation. Some reports have demonstrated that the neutrophilic inflammatory response is essential for controlling Ehrlich tumor growth, and the high influx of these cells promotes tumor development.[30,31] This effect is probably related with the angiogenesis and growth factors induced by inflammation that are necessary for the tumor growth. The Ehrlich solid tumor implantation induces per se a local inflammatory reaction, with increasing vascular permeability, which results in an intense edema formation, cellular migration and a progressive ascitic fluid formation, which is essential for tumor growth, since this fluid constitutes the direct nutritional source for tumor cells.[32,33]

The preliminary in vivo study with compound **4**, concerning the acute toxicological effects, determined the doses of 30 mg/kg as the higher one to be used on the following in vivo experiments, based on the fact that this dose did not produced toxicological effects (not shown). Therefore, doses of 10 and 30 mg/kg of compound **4** were determined to be evaluated on the Ehrlich solid carcinoma.

The results of the Ehrlich carcinoma assay for compound **4** demonstrate its systemic effectiveness as a tumor growth inhibitor. This assertion is supported by the fact that the compound given by intraperitoneal route (ip) every 3 days during the 15-days period of experiment, was capable of reducing tumor growth (such as observed for the positive control 5-Fluoruracil) when compared to the negative control group (vehicle). It was observed that in the initial phase of the tumor growth (first 3 days after inoculation), representing the inflammation process involving several mediators, such as prostaglandins, leukotrienes, histamine and bradykinin,[34] the treatment with the 30 mg/kg doses of compound **4** reduced the paw edema compared to control group ($^{*}P$ <0.05).

The result discussed above suggests that compound **4** may act decreasing pro-inflammatory factors. The inflammatory factors promote tumor growth due to their role in the relation between tumor and stroma cells thus establishing a tumor microenvironment which facilitates angiogenesis and evades the immune system attack. Also, leukotrienes modulate proliferation, tumor differentiation, apoptosis and interfere with migration, and invasion of the tumor cells. In the tumor microenvironment, cells of the immune system and endothelium are recruited to produce more pro-inflammatory mediators, including eicosanoids, growth, and angiogenic factors.[34]

Starting from the ninth day trial of the experiment, the results of the present study also showed that, during the exponential proliferative phase of the tumor growth with cell proliferation, production of tumor and inflammation factors, compound **4** was capable of decrease the tumor growth. It derives from the fact that the positive control 5-FU (10 mg/kg) and both doses (10 and 30 mg/kg) of the compound reduced the tumor volume ($^{*}P$ <0.05) of the mice paw compared to the control group (vehicle). Moreover, compound **4** (30 mg/kg) and 5-FU maintained the activity until the end of the experiment, demonstrating that in the 12-day ($^{*}P$ <0.05; $^{**}P$ <0.01) and 15-day ($^{*}P$ <0.05; $^{***}P$ <0.001) evaluation of tumor growth, both groups significantly reduced the mice paw edema (Fig. 1).

The complexity of the tumor biology related to its interaction with the associated stroma, often leads to failure when drugs with a good in vitro profile enter to in vivo experimentation.[35] However, it is not the case of compound **4,** which displayed a promising in vivo activity. Considering the complex processes involved in Ehrlich carcinoma growth, the effectiveness of compound **4** may be related to several mechanisms, such as the cell proliferation or in the inflammatory responses that facilitate tumor growth. Thus, further studies are necessary to clarify the specific mechanism of action of compound **4**.

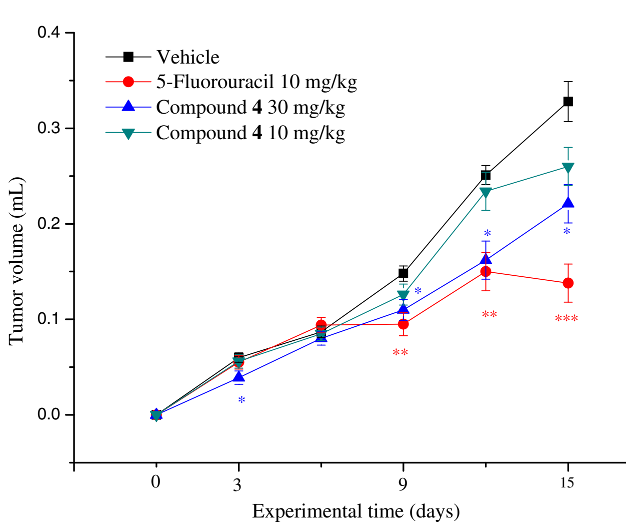

Figure 1. Effect of compound **4** on Ehrlich solid tumor expressed as tumor volume (mL) per day of treatment. Treatment with **4** at 30 mg/kg doses reduced tumor volume starting at the third day (acute inflammatory response, $^{*}P$ <0.05), and from the ninth until the end of experiment (15° day, $^{*}P$ <0.05), which represents the tumor growth itself. A dose of 10 mg/kg only reduced tumor volume on the ninth day ($^{*}P$ <0.05). 5-Fluorouracil was the positive control and vehicle was saline 0.9%.

3. Conclusion

In summary, in this work we have synthesized and evaluated the antitumor activity of a number of β-carboline 3-N-substituted-carbohydrazides against a panel of human cancer cell lines. In general, we have demonstrated that the presence of a carbohydrazide moiety at C-3 of a 1-substituted-phenyl β-carboline nucleus can enhance the antitumor activity. The β-carboline 3-*N*-benzylidene-carbohydrazides presented greater activity than the 3-*N*-alkylidene-carbohydrazide analogues. The N^9-methylation of the β-carboline 3-benzylidene-carbohydrazides resulted in a decrease of antitumor activity.

The derivative **4** displayed significant activity towards all cell lines tested, being highly active against renal (786-0) cell lines with an IC_{50} of 0.04 μM. The results of the Ehrlich carcinoma assay for compound **4** demonstrate its systemic effectiveness, as a tumor growth inhibitor. The treatment with the 30 mg/kg doses of compound **4** reduced the Ehrlich tumor volume of the mice paw, suggesting that its may act decreasing pro-inflammatory factors.

4. Experimental

4.1. General

All reagents were purchased from commercial suppliers. The reactions were monitored by thin layer chromatography conducted on Merck TLC plates (Silica Gel 60 F_{254}). ^{1}H and ^{13}C spectra were recorded on a Varian spectrometer model Mercury Plus BB at 300 MHz and 75 MHz, respectively. Mass spectra (MS) were recorded on a Thermoelectron Corporation Focus-DSQ II spectrometer. IR spectra were obtained on a BOMEM spectrometer model MB-100.

4.2. General procedure for the synthesis of *N*′-benzylidene-1-(substituted-phenyl)-β-carboline-3-carbohydrazides (3–26)

The synthesis and spectral characterization of the N-(substituted-benzylidene)-β-carboline-3-carbohydrazides (**3–26**) were described in a previous work.[28]

4.3. General procedure for the synthesis of *N*′-alkylidene-1-(substituted-phenyl)-β-carboline-3-carbohydrazides (27–50)

To a solution of the carbohydrazides **2a–h** (4.0 mmol) in EtOH (20 mL) was added cyclohexanone, cyclopentanone and propanone (3 equiv), and the resulting solution was refluxed for 30 h. The reaction mixture was then cooled and kept at 0 °C for 3 h. The resulting solids were filtered off and washed with cold EtOH to furnish the products in a pure form.

4.3.1. *N*′-Cyclohexylidene-1-(4-methoxyphenyl)-β-carboline-3-carbohydrazide (27)

Yield: 82%; mp 267–269 °C; IR (KBr) ν_{max}: 3227 (N-H), 1609 (C=N), 1684 (C=O), 1558–1347 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ 3:1): δ 1.68–1.78 (m, 6H, H_2-3″, H_2-4″ and H_2-5″), 2.47 (t, *J* = 6.3 Hz, 2H, H_2-6″), 2.53 (t, *J* = 6.3 Hz, 2H, H_2-2″), 3.93 (s, 3H), 7.17 (d, *J* = 8.7 Hz, 2H, H-3′ and H-5′), 7.37 (t, *J* = 7.8 Hz, 1H, H-6), 7.54–7.62 (m, 2H, H-7 and H-8), 7.93 (d, *J* = 8.7 Hz, 2H, H-2′ and H-6′), 8.23 (d, *J* = 7.8 Hz, 1H, H-5), 8.71 (s, 1H, NH), 8.93 (s, 1H, H-4), 11.11 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 25.5 (CH_2, C-3″), 26.0 (CH_2, C-5″), 26.9 (CH_2, C-6″), 27.0 (CH_2, C-4″), 35.5 (CH_2, C-2″), 55.4 (O–CH_3), 112.2 (CH, C-4), 113.5 (CH,C-8), 114.4 (CH, C-3′ and C-5′), 120.6 (CH, C-6), 121.7 (CH, C-5), 128.7 (CH, C-7), 129.5 (CH, C-2′ and C-6′), 130.1 (C, C-4b), 130.3 (C, C-3), 134.8 (C, C-1′), 138.2 (C, C-9a), 140.9 (C, C-8a), 141.4 (C, C-1), 160.4 (C, C-4′), 161.4 (C=N), 162.6 (C=O); MS *m/z* (%): 412.05 (M^+, 85), 369.00 (53), 273.00 (98), 272.97 (100), 257.95 (50).

4.3.2. *N*′-Cyclohexylidene-1-phenyl-β-carboline-3-carbohydrazide (28)

Yield: 85%; mp 284–286 °C; IR (KBr) ν_{max}: 3154 (N-H), 1623 (C=N), 1676 (C=O), 1556-1345 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ 3:1): δ 1.65–1.82 (m, 6H, H_2-3″, H_2-4″ and H_2-5″), 2.46 (t, *J* = 6,3 Hz, 2H, H_2-6″), 2.52 (t, *J* = 6.3 Hz, 2H, H_2-2″), 7.38 (dd, *J* = 6.3 Hz, *J* = 1.5 Hz, 1H, H-6), 7.54–7.60 (m, H-3′, H-4′, H-5″, H-7 and H-8), 7.99 (dd, *J* = 7.6 Hz, *J* = 1,4 Hz, 2H, H-2′ and H-6′), 8.23 (d, *J* = 7.8 Hz, 1H, H-5), 8.79 (s, 1H, N H), 9.02 (s, 1H, H-4), 11.10 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz,): δ 25.2 (CH_2, C-3″), 26.0 (CH_2, C-5″), 26.9 (CH_2, C-6″), 27.0 (CH_2, C-4″), 35.3 (CH_2, C-2″), 112.2 (CH, C-4), 113.9 (CH, C-8), 120.6 (CH, C-6), 121.8 (CH, C-5), 128.2 (2CH, C-2′ and C-6′), 128.7 (CH, C-7), 129.0 (2CH, C-3′ and C-5′), 129.1 (C, C-4′), 130.5 (C, C-4a), 135.1 (C, C-1′), 137.9 (C, C-9a), 138.6 (C, C-3), 141.1 (C, C-8a), 141.3 (C, C-1), 161.9 (C=N), 162.4 (C=O); MS *m/z* (%): 382.05 (M^+, 48), 243.99 (63), 242.98 (100).

4.3.3. *N*′-Cyclohexylidene-1-(3-nitrophenyl)-β-carboline-3-carbohydrazide (29)

Yield: 88%; mp 279–281 °C; IR (KBr) ν_{max}: 3250 (N-H), 1624 (C=N), 1667 (C=O), 1557–1345 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ 3:1): δ 1.70–1.79 (m, 6H, H_2-3″, H_2-4″ and H_2-5″), 2.48–2.51 (m, 2H, H_2-6″), 2.51–2.55 (m, 2H, H_2-2″), 7.42 (dd, *J* = 8.1 Hz, *J* = 1.6 Hz, 1H, H-6), 7.60–7.68 (m, 2H, H-7 and H-8), 7.84 (t, *J* = 7.9 Hz, 1H, H-5′), 8.25 (d, *J* = 8.1 HZ, 1H, H-5), 8.36–8.41 (m, 2H, H-4′ and H-6′), 8.84 (s, 1H, NH), 8.93 (t, *J* = 1.8 Hz, 1H, H-2′), 9.08 (s, 1H, H-4), 11.04 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 25.4 (CH_2, C-3″), 25.9 (CH_2, C-5″), 26.8 (CH_2, C-6″), 27.0 (CH_2, C-4″), 35.2 (CH_2, C-2″), 112.4 (CH, C-8), 114.6 (CH, C-4), 120.9 (CH, C-6), 121.5 (C, C-4b), 121.7 (CH, C-5), 123.4 (CH, C-2′), 129.9 (CH, C-4′), 129.9 (CH, C-6′), 131.5 (C, C-4a), 133.9 (CH, C-7), 133.9 (C, C-1′), 133.9 (CH, C-5′), 134.9 (C, C-9a), 138.5 (C, C-3), 139.5 (C, C-8a), 141.7 (C, C-1), 148.7 (C, C-3′), 162.8 (C=N), 162.8 (C=O); MS *m/z* (%): 427.07 M^+, 58), 384.01 (38), 289.00 (60), 241.98 (100).

4.3.4. *N*′-Cyclohexylidene-1-(3-hydroxy-4-methoxyphenyl)-β-carboline-3-carbohydrazide (30)

Yield: 90%; mp 288–290 °C; IR (KBr) ν_{max}: 3321 (N-H), 1623 (C=N), 1680 (C=O), 1562–1349 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6/$CDCl_3$ 3:1): δ 1.73–1.79 (m, 6H, H_2-3″, H_2-4″ and H_2-5″), 2.51 (*t*, *J* = 6.0 Hz, 4H, H_2-2″ and H_2-6″), 4.01 (s, 3H, OMe), 7.35 (t, *J* = 6.6 Hz, 1H, H-6), 7.53–7.61 (m, 4H, H-2′, H-5′, H-6′ and H-7′), 7.63 (d, *J* = 7.8 Hz, 1H, H-8), 8.23 (d, *J* = 8.1 Hz, 1H, H-5), 8.88 (s, 1H, H-4); ^{13}C NMR (75.5 MHz): δ 25.4 (CH_2, C-4″), 26.0 (CH_2, C-5″), 26.8 (CH_2, C-3″), 26.9 (CH_2, C-6″), 35.2 (CH_2, C-2″), 55.7 (C, C-OMe), 111.4 (CH, C-2′), 112.1 (CH, C-8), 113.4 (CH, C-4), 115.2 (CH, C-5′), 120.5 (CH, C-6), 121.1 (CH, C-6′), 121.7 (CH, C-5), 121.8 (C, C-4b), 128.6 (CH, C-7), 129.7 (C, C-1′), 130.3 (C, C-4a), 134.9 (C, C-9a), 138.3 (C, C-3), 141.3 (C, C-8a), 141.4 (C, C-1), 147.2 (C, C-4′), 147.9 (C, C-3′), 161.9 (C=N), 162.1 (C=O); MS *m/z* (%): 428.08 (M^+, 100), 385.03 (72), 290.02 (98), 256.98 (90).

4.3.5. *N*′-Cyclohexylidene-1-(4-hydroxyphenyl)-β-carboline-3-carbohydrazide (31)

Yield: 87%; mp >300 °C; IR (KBr) ν_{max}: 3446 (N-H), 1611 (C=N), 1659 (C=O), 1557–1347 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6/$CDCl_3$ 3:1): δ 1.71–1.80 (m, 6H, H_2-3″, $H_2$4″ and H_2-5″), 2.47–2.52 (m, 4H, H_2-2″ and H_2-6″), 7.08 (d, *J* = 8.4 Hz, 2H, H-3′ and H-5′), 7.33 (td, *J* = 8.1 Hz, *J* = 1.4 Hz, 1H, H-6), 7.56 (t, *J* = 8.1 Hz, 1H, H-7), 7.60 (d, *J* = 8.1 Hz, 1H, H-8), 7.88 (d, *J* = 8.4 Hz, 2H, H-2′ and H-6′), 8.21 (d, *J* = 8.1 Hz, 1H, H-5), 8.86 (s, 1H, H-4); ^{13}C NMR (75.5 MHz): δ 25.5 (CH_2, C-3″), 26.1 (CH_2, C-5″), 27.0 (CH_2, C-6″), 27.1 (CH_2, C-4″), 35.3 (CH_2, C-2″), 112.2 (CH, C-4), 113.4 (CH, C-8), 115.9 (2 CH, C-3′and C-5′), 120.6 (CH, C-6), 121.8 (C, C-4a), 121.9 (CH, C-5), 128.6 (CH, C-7), 129.4 (C, C-4b), 129.7 (2 CH, C-2′ and C-6′), 130.2 (C, C-3), 135.1 (C, C-1′), 138.4 (C, C-9a), 141.4 (C, C-8a), 141.7 (C, C-1), 158.1 (C, C-4′), 162.2 (C=N), 162.8 (C=O); MS *m/z* (%): 398.07 (M^+, 50), 355.02 (28), 258.99 (100).

4.3.6. *N*′-Cyclohexylidene-1-[4-(dimethylamino)phenyl]-β-carboline-3-carbohydrazide (32)

Yield: 90%; mp >300 °C; IR (KBr) ν_{max}: 1623 (C=N), 1676 (C=O), 1554–1347 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ 3:1): δ 1.68–1.89 (m, 6H, H_2-3″, H_2-4″ and H_2-5″), 2.49 (t, *J* = 6.3 Hz, 2H, H_2-6″), 2.53 (t, *J* = 6.3 Hz, 2H, H_2-2″), 3.10 (s, 6H, $N(CH_3)_2$), 6.94 (d, *J* = 8.8 Hz, 2H, H-3′ and H-5′), 7.36 (*t*, *J* = 7.8 Hz, 1H, H-6), 7.52–7.61 (m, 1H, H-7), 7.59 (d, *J* = 7.8 Hz, 1H, H-8), 7.89 (d, *J* = 8.8 Hz, 2H, H-2′ and H-6′), 8.21 (d, *J* = 7.8 Hz, 1H, H-5), 8.72 (s, 1H, NH), 8.93 (s, 1H, H-4), 11.10 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 25.5 (CH_2, C-3″), 26.0 (CH_2, C-5″), 26.9 (CH_2, C-6″), 27.0, (CH_2, C-4″) 35.3 (CH_2, C-2″), 40.3 (N–$(CH_3)_2$), 112.1 (CH, C-4), 112.5 (2CH, C-3′ and C-5′), 112.9 (CH, C-8), 120.5 (CH, C-6), 121.8 (CH, C-5), 122.0 (C, C-4b), 125.6 (C, C-1′), 128.4 (CH, C-7), 129.0 (2 CH,C-2′ and C-6′), 129.9 (C, C-4a), 134.7 (C, C-9a), 138.5 (C, C-8a), 141.1 (C, C-3), 141.8 (C, C-1), 151.0 (C, C-4′), 162.1 (C=N), 162.1 (C=O); MS *m/z* (%): 425.11 (M^+, 92), 382.05 (63), 286.02 (100), 241.97 (82).

4.3.7. *N*′-Cyclohexylidene-1-(4-nitrophenyl)-β-carboline-3-carbohydrazide (33)

Yield: 90%; mp 292–295 °C; IR (KBr) ν_{max}: 3308 (N-H), 1628 (C=N), 1669 (C=O), 1557–1341 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.65–1.68 (m, 6H, H_2-3″, H_2-4″ and H_2-5″), 2.36–2.50 (m, 4H, H_2-2″ and H_2-6″), 7.36 (t, *J* = 8.1 Hz, 1H, H-6), 7.64 (d, *J* = 8.1 Hz, 1H, H-8), 7.69 (t, *J* = 8.1 Hz, 1H, H-7), 8.43–8.49 (m, 3H, H-3′, H-5′ and H-5), 8.49–8.51 (m, 2H, H-2′and H-6′), 8.79 (s, 1H, NH), 9.00 (s, 1H, H-4), 12.12 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ25.0 (CH_2, C-3″), 25.5 (CH_2, C-5″), 26.6 (CH_2, C-6″), 27.0 (CH_2, C-4″), 35.0 (CH_2, C-2″), 112.6 (CH, C-8), 114.6 (CH, C-4), 120.6 (CH, C-6), 121.0 (C, C-4b), 122.4 (CH, C-5), 123.9 (2CH, C-3′ and C-5′), 129.1 (CH, C-7), 130.0 (2CH, C-2′ and C-6′), 130.7 (C, C-4a), 134.6 (C, C-1′), 138.0 (C, C-9a), 139.5 (C, C-3), 141.7 (C, C-8a), 143.5 (C, C-1), 147.5 (C, C-4′), 160.0 (C=N), 163.2 (C=O); MS *m/z* (%): 427.07 (M^+, 60), 289.00 (65), 241.98 (100).

4.3.8. *N*′-Cyclohexylidene-1-(2-chlorophenyl)-β-carboline-3-carbohydrazide (34)

Yield: 78%; mp 276–279 °C; IR (KBr) ν_{max}: 3312 (N-H), 1623 (C=N), 1673 (C=O), 1596–1347 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.62–1.91 (m, 6H, H_2-3″, H_2-4″ and H_2-5″), 2.42 (t, *J* = 6.3 Hz, 2H, H_2-6″), 2.49 (t, *J* = 6.3 Hz, 2H, H_2-2″), 7.37 (dd, *J* = 8.1 Hz, *J* = 1.2 Hz, 1H, H-6), 7.47–7.52 (m, 2H, H-3′ and H-6″), 7.55–7.71 (m, 4H, H-4′, H-5′, H-7 and H-8), 8.23 (d, *J* = 8.1 Hz, 1H, H-5), 8.58 (s, 1H, NH), 9.06 (s, 1H, H-4), 10.97 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 25.8 (CH_2, C-3″), 26.2 (CH_2, C-5″), 27.1 (CH_2, C-4″), 27.1 (CH_2, C-6″), 35.8 (CH_2, C-2″), 112.0 (CH, C-8), 115.0 (CH, C-4), 121.3 (CH, C-6), 122.2 (C, C-4b), 122.5 (CH, C-5), 127.6 (CH, C-7), 129.3 (CH, C-5′), 130.6 (CH, C-3′), 130.8 (CH, C-6′), 131.8 (CH, C-4′), 133.2 (C, C-2′), 135.6 (C, C-1′), 136.2 (C, C-9a), 139.2 (C, C-3), 139.8 (C, C-8a), 140.8 (C, C-1), 161.3 (C=N), 161.6 (C=O); MS *m/z* (%): 416.05 (M^+, 50), 277.98 (70), 241.98 (100).

4.3.9. *N*′-Cyclopentylidene-1-(4-methoxyphenyl)-β-carboline-3-carbohydrazide (35)

Yield: 78%; mp 265–267 °C; IR (KBr) ν_{max}: 3238 (NH), 1623 (C=N), 1678 (C=O), 1559–1345 (C=C) cm^{-1}; ^{1}H NMR (300 MHz,

$CDCl_3/CD_3OD$ 3:1): δ 1.84 (quint, J = 6.8 Hz, 2H, H_2-4″), 1.96 (quint, J = 6.8 Hz, 2H, H_2-3″), 2.47 (t, J = 6.8 Hz, 2H, H_2-5″), 2.59 (t; J = 6.8 Hz, 2H, H_2-2″), 3.98 (s, 3H, OCH_3), 7.16 (d, J = 8.1 Hz, 2H, H-3′and H-5″), 7.33 (dd, J = 7.5 Hz, J = 1,0 Hz, 1H, H-6), 7.57 (dd, J = 7.5 Hz, J = 1,0 Hz, 1H, H-7), 7.62 (d, J = 7.5 Hz, 1H, H-8), 7.97 (d, J = 8.1 Hz, 2H, H-2′ and H-6′), 8.19 (d, J = 7.5 Hz, 1H, H-5), 8.84 (s, 1H, H-4); ^{13}C NMR (75.5 MHz): δ 24.7 (CH_2, C-4″), 24.8 (CH_2, C-3″), 27.5 (CH_2, C-5″), 33.2 (CH_2, C-2″), 55.4, 112.2 (CH, C-8), 113.5 (CH, C-4), 114.4 (2CH, C-3′ and C5′), 120.6 (CH, C-6), 121.8 (CH, C-5), 121.9 (C, C-4b), 128.6 (CH, C-7), 129.5 (2CH, C-2′ and 6′), 130.3 (C, C-1′), 130.4 (C, C-4a), 134.9 (C, C-9a), 138.3 (C, C-3), 141.0 (C, C-8a), 141.3 (C, C-1), 160.3 (C, C-4′), 161.6 (C=N), 168.1 (C=O); MS m/z (%): 398.09 (M^+, 83), 369.05 (75), 273.02 (100).

4.3.10. N′-Cyclopentylidene-1-phenyl-β-carboline-3-carbohydrazide (36)

Yield: 80%; mp 268–271 °C; IR (KBr) ν_{max}: 1624 (C=N), 1690 (C=O), 1557–1344 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6/$CDCl_3$ 3:1): δ 1.80 (quint., J = 6.7 Hz, 4H, H_2-3″ and H_2-4″), 2.48–2.54 (m, 4H, H_2-2″ and H_2-5″), 7.31 (t, J = 7.8 Hz, 1H, H-6), 7.53–7.71 (m, 5H, H-7, H-8, H-3′, H-4′ and H-5′), 8.14–8.10 (m, 2H, H-2′ and H-6′), 8.34 (d, J = 7.8 Hz, 1H, H-5), 8.90 (s, 1H, H-4), 10.79 (s, 1H, NH), 11.80 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 24.2 (CH_2, C-4″), 24.3 (CH_2, C-3″), 26.8 (CH_2, C-5″), 32.9 (CH_2, C-2″), 112.5 (CH, C-8), 113.2 (CH, C-4), 120.0(CH, C-6), 121.0 (C, C-4b), 121.6 (CH, C-5), 128.2 (CH, C-4′), 128.3 (2CH, C-2′ and C-6′), 128.6 (2CH, C-3′and C-5′), 128.7 (CH, C-7), 129.9 (C, C-4a), 134.4 (C, C-1′), 137.4 (C, C-9a), 138.3 (C, C-3), 140.4 (C, C-8a), 141.5 (C, C-1), 159.9 (C=N), 166.4 (C=O); MS m/z (%): 368.07 (M^+, 38), 339.04 (32), 243.00 (100).

4.3.11. N′-Cyclopentylidene-1-(3-nitrophenyl)-β-carboline-3-carbohydrazide (37)

Yield: 77%; mp 300 °C, decomp.; IR (KBr) ν_{max}: 3214 (N-H), 1624 (C=N), 1683 (C=O), 1561–1349 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ 3:1): δ 1.82–1.89 (m, 2H, H_2-4″), 191–1.98 (m, 2H, H_2-3″), 2.45 (t, J = 6.0 Hz, 2H, H_2-5″), 2.61 (t, J = 6.0 Hz, 2H, H_2-2″), 7.58–7.62 (m, 2H, H-7 and H-8), 7.83 (t, J = 7.8 Hz, 1H, H-4′), 8.37–8.43 (m, 2H, H-5′ and H-6′), 8.86–8.87 (m, 1H, H-2′), 8.18 (d, J = 7.8 Hz, 1H, H-5), 8.91 (s, 1H, H-4); ^{13}C NMR (75.5 MHz): δ 24.6 (CH_2, C-3″), 24.6 (CH_2, C-4″), 27.4 (CH_2, C-5″), 33.1 (CH_2, C-2″), 112.3 (CH, C-8), 114.5 (CH, C-4), 120.8 (CH, C-6), 121.4 (C, C-4b), 121.6 (CH, C-5), 123.3 (CH, C-2′), 123.4 (CH, C-6′), 129.0 (CH, C-4′), 129.9 (CH, C-7), 131.3 (C, C-4a), 133.8 (CH, C-5′), 135.0 (C, C-1′), 137.9 (C, C-9a), 138.1 (C, C-3), 139.4 (C, C-8a), 141.7 (C, C-1), 148.6 (C, C-3′), 161.1 (C=N), 168.7 (C=O); MS m/z (%): 413.06 (M^+, 38), 384.03 (42), 288.00 (50), 242.00 (100) .

4.3.12. N′-Cyclopentylidene-1-(3-hydroxy-4-methoxyphenyl)-β-carboline-3-carbohydrazide (38)

Yield: 77%; mp 296–297 °C; IR (KBr) ν_{max}: 1623 (C=N), 1682 (C=O), 1598–1346 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6/$CDCl_3$ 3:1): δ 1.82 (quint., J = 6.9 Hz, 2H, H_2-4″), 1.91 (quint., J = 6.9 Hz, 2H, H_2-3″), 2.49–2.57 (m, 4H, H_2-2″ and H_2-5″), 3.98 (s, 3H, OCH_3), 7.06 (d, J = 8.1 Hz, 1H, H-5′), 7.30 (t, J = 7.8 Hz, 1H, H-6), 7.53–7.59 (m, 2H, H-6′ and H-7), 7.68–7.71 (m, 2H, H-2′ and H-8), 8.33 (d, J = 7.8 Hz, 1H, H-5), 8.82 (s, 1H, H-4), 9.37 (s, 1H, OH), 10.87 (s, 1H, NH), 11.82 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 24.1 (CH_2, C-4″), 24.2 (CH_2, C-3″), 26.8 (CH_2, C-5″), 32.7 (CH_2, C-2″), 55.3 (OCH_3), 111.8 (CH, C-2′), 112.4 (CH, C-8), 112.5 (CH, C-5′), 115.3 (CH, C-4), 119.9 (CH, C-6), 121.0 (C, C-4b), 121.1 (CH, C-5), 121.5 (CH, C-6′), 128.1 (C, C-4a), 128.5 (C, C-1′), 129.5 (C, C-7), 134.0 (C, C-9a), 138.1 (C, C-3), 140.8 (C, C-8a), 141.3, (C, C-1) 147.7 (C, C-4′), 147.8 (C, C-3′), 159.9 (C=N), 166.2 (C=O); MS m/z (%): 414.06 (M^+, 100), 385.02 (95), 290.00 (95), 256.97 (90).

4.3.13. N′-Cyclopentylidene-1-(4-hydroxyphenyl)-β-carboline-3-carbohydrazide (39)

Yield: 80%; mp 300 °C, decomp; IR (KBr) ν_{max}: 1609 (C=N), 1656 (C=O), 1446–1346 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6/$CDCl_3$ 3:1): δ 1.87 (quint., J = 6.9 Hz, 2H, H_2-4″), 1.96 (quint., J = 6.9 Hz, 2H, H_2-3″), 2.51 (t, J = 6.9 Hz, 2H, H_2-5″), 2.61 (t, J = 6.9 Hz, 2H, H_2-2″), 7.08 (d, J = 8.4 Hz, 2H, H-3′ and H-5′), 7.30 (t, J = 7.5 Hz, 1H, H-6), 7.55 (t, J = 7.5 Hz, 1H, H-7), 7.69 (d, J = 7.5 Hz, 1H, H-8), 7.88 (d, J = 8.4 Hz, 2H, H-2′ and H-6′), 8.24 (d, J = 7.5 Hz, 1H, H-5), 8.89 (s, 1H, H-4), 10.81 (s, 1H, NH), 11.76 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 24.2 (CH_2, C-4″), 24.3 (CH_2, C-3″), 26.8 (CH_2, C-5″), 32.8 (CH_2, C-2″), 112.4 (CH, C-4), 112.5 (CH, C-8), 115.5 (2CH, C-3′ and 5′), 119.9 (CH, C-6), 121.1 (C, C-4b), 125.4 (CH, C-5), 128.1 (CH, C-7), 128.2 (C, C-4a), 129.5 (C, C-9a), 129.5 (C-2′ and C-6′), 134.0 (C, C-1′), 138.1 (C, C-3), 140.9 (C, C-8a), 141.3 (C, C-1), 158.3 (C, C-4′), 160.0 (C=N), 166.3 (C=O); MS m/z (%): 384.08 (M^+, 47), 355.02 ((40), 259.00 (100).

4.3.14. N′-Cyclopentylidene-1-[4-(dimethylamino)phenyl]-β-carboline-3-carbohydrazide (40)

Yield: 85%; mp 269–270 °C; IR (KBr) ν_{max}: 1622 (C=N), 1675 (C=O), 1555–1347 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ 3:1): δ 1.84 (quint., J = 6.9 Hz, 2H, H_2-4″), 1.95 (quint., J = 6.9 Hz, 2H, H_2-3″), 2.48 (t, J = 6.9 Hz, 2H, H_2-5″), 2.59 (t, J = 6.9 Hz, 2H, H_2-2″), 3.09 (s, 6H, $(CH_3)_2$), 6.95 (d, J = 9.0 Hz, 2H, H-3′ and H-5′), 7.33–7.36 (m), 7.54–7,61 (m, 2H, H-7 and H-8), 7.92 (d, J = 9.0 Hz, 2H, H-2′ and H-6′), 8.20 (d, J = 7.8 Hz, 1H, H-5), 8.82 (s, 1H, H-4); ^{13}C NMR (75.5 MHz): δ 24.6 (CH_2, C-4″), 24.7 (CH_2, C-3″), 27.4 (CH_2, C-5″), 33.1 (CH_2, C-2″), 40.1 $(CH_3)_2$, 112.1 (CH, C-4), 112.4 (2CH, C-3′ and C-5′), 112.7 (CH, C-8), 120.3 (CH, C-6), 121.5 (C, C-4b), 121.8 (CH, C-5), 125.4 (C, C-4a), 128.3 (CH, C-7), 128.9 (2CH, C-2′ and C-6′), 129.9 (C, C-1′), 134.7 (C, C-9a), 137.9 (C, C-3), 141.2 (C, C-8a), 141.9 (C, C-1), 151.0 (C, C-4′), 161.9 (C=N), 168.2 (C=O); MS m/z (%): 411.12 (M^+, 92), 382.07 (87), 286.07 (100), 242 (85).

4.3.15. N′-Cyclopentylidene-1-(4-nitrophenyl)-β-carboline-3-carbohydrazide (41)

Yield: 88%; mp 291–293 °C; IR (KBr) ν_{max}: 1624 (C=N), 1682 (C=O), 1555–1341 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.75 (quint, J = 6.3 Hz, 2H, H_2-4″), 1.85 (quint, J = 6.3 Hz, 2H, H_2-3″), 2.44–2.51 (m, 2H, H_2-2″), 2.44–2.51 (m, 2H, H_2-5″), 7.36 (t, J = 7.8 Hz, 1H, H-6), 7.65 (t, J = 7.8 Hz, 1H, H-7), 7.72 (d, J = 7.8 Hz, 1H, H-8), 8.44 (d, J = 9.0 Hz, 2H, H-3′ and H-5′), 8.46 (d, J = 7.8 Hz, 1H, H-5), 8.50 (d, J = 9.0 Hz, 2H, H-2′ and H-6′), 9.03 (s, 1H, H-4), 10.7 (s, 1H, NH), 12.1 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 24.3 (CH_2, C-4″), 24.4 (CH_2, C-3″), 27.1 (CH_2, C-5″), 32.9 (CH_2, C-2″), 112.6 (CH, C-8), 114.6 (CH, C-4), 120.6 (CH, C-6), 121.0 (CH, C-4b), 122.4 (CH, C-5), 124.0 (2CH, C-3′ and C-5′), 129.2 (CH, C-7), 129.9 (2CH, C-2′ and C-6′), 130.8 (C, C-4a), 134.7 (C, C-1′), 138.0 (C, C-9a), 139.1 (C, C-3), 141.7 (C, C-8a), 143.5 (C, C-1), 147.5 (C, C-4′), 159.5 (C=N), 167.7 (C=O); MS m/z (%): 413.06 (M^+, 53), 384.03 (45), 288.00 (58), 258.02 (58), 242.00 (100).

4.3.16. N′-Cyclopentylidene-1-(2-chlorophenyl)-β-carboline-3-carbohydrazide (42)

Yield: 77%; mp 280–282 °C; IR (KBr) ν_{max}: 1623 (C=N), 1669 (C=O), 1563–1345 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.79 (quint., J = 7.2 Hz, 2H, H_2-4″), 1.89 (quint., J = 7.2 Hz, 2H, H_2-3″), 2.41 (t, J = 7.2 Hz, 2H, H_2-5″), 2.56 (t, J = 7.2 Hz, 2H, H_2-2″), 7.39 (ddd; J = 7.8 Hz, J = 6.7 Hz, J = 1.5 Hz, 1H, H-6), 7.46–7.51 (m, 2H, H-7 and H-8), 7.55–7.71 (m, 4H, H-3′, H-4′ H-5′and H-6′), 8.22 (d, J = 7.8 Hz, 1H, H-5), 8.67 (s, 1H, NH), 9.05 (s, 1H, H-4), 10.67 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 24.9 (CH_2, C-4″), 25.0 (CH_2, C-3″), 27.6 (CH_2, C-5″), 33.6 (CH_2, C-2″), 112.0 (CH, C-8), 115.0 (CH, C-4), 121.3 (CH, C-6), 122.2 (C, C-4b), 122.4 (CH, C-5), 127.6 (CH, C-7), 129.3 (CH, C-5′), 130.5 (CH, C-3′), 130.6 (C, C-4a), 130.8 (CH, C-6′), 131.7 (CH, C-4′), 133.2 (C, C-2′), 135.6 (C, C-1′), 136.2 (C, C-9a), 139.2 (C, C-3), 139.5 (C, C-8a), 140.8 (C, C-1), 161.1 (C=N), 167.4 (C=O); MS *m/z* (%): 401.99 (M^+, 50), 276.94 (67), 241.97 (100).

4.3.17. *N*′-2-Propylidene-1-(4-methoxyphenyl)-β-carboline-3-carbohydrazide (43)

Yield: 80%; mp 285–288 °C; IR (KBr) ν_{max}: 1624 (C=N), 1686 (C=O), 1561–1346 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.07 (s, 3H, H_3-3″), 2.20 (s, 3H, H_3-2″), 3.94 (s, 3H, OCH_3), 7.16 (d, *J* = 8.7 Hz, 2H, H-3′ and H-5′), 7.37 (dd, *J* = 8.1 Hz, *J* = 1.5 Hz, 1H, H-6), 7.54–7.63 (m, 2H, H-7 and H-8), 7.93 (d, *J* = 8.7 Hz, 2H, H-2′ and H-6′), 8.22 (d, *J* = 8.1 Hz, 1H, H-5), 8.73 (s, 1H, NH), 8.98 (s, 1H, H-4), 11.03 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 16.8 (CH_3, C-3″), 25.8 (CH_3, C-2″), 55.7 (OCH_3), 111.9 (CH, C-8), 114.1 (CH, C-4), 115.0 (2CH, C-3′ and C-5′), 121.3 (CH, C-6), 122.3 (C, C-4b), 122.5 (CH, C-5), 129.1 (CH, C-7), 129.4 (2CH,C-2′ and C-6′), 129.6 (C, C-4a), 130.6 (C, C-3), 130.7 (C, C-1′), 134.9 (C, C-9a), 140.0 (C, C-8a), 140.7 (C, C-1), 154.8 (C=N), 160.6 (C-4′), 161.3 (C=O); MS *m/z* (%): 372.04 (M^+, 55), 357.02 (57), 272.99 (100).

4.3.18. *N*′-2-Propylidene-1-phenyl-β-carboline-3-carbohydrazide (44)

Yield: 82%; mp 284–286 °C; IR (KBr) ν_{max}: 1625 (C=N), 1678 (C=O), 1560–1344 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.05 (s, 3H, H_3-3″), 2.18 (s, 3H, H_3-2″), 7.37 (dd, *J* = 7.8 Hz, *J* = 1.8 Hz, 1H, H-6), 7.52–7.57 (m, 2H, H-7 and H-8), 7.59–7.66 (m, 2H, H-3′ and H-5′), 7.97–8.00 (m, 2H, H-2′ and H-6′), 8.21 (d, *J* = 7,8 Hz, 1H, H-5), 8.87 (s, 1H, NH), 9.00 (s, 1H, H-4), 11.01 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 16.7 (CH_3, C-3″), 25.7 (CH_3, C-2″), 112.2 (CH, C-8), 114.3 (CH, C-4), 121.2 (CH, C-6), 122.2 (C, C-4b), 122.3 (CH, C-5), 128.2 (2CH, C-2′ and C-6′), 129.1 (CH, C-7), 129.3 (CH, C-4′), 129.4 (2CH, C-3′ and C-5′), 130.8 (C, C-4a), 135.1 (C, C-1′), 138.0 (C, C-9a), 139.6 (C, C-3), 140.9 (C, C-8a), 141.0 (C, C-1), 154.9 (C=N), 161.3 (C=O); MS *m/z* (%): 342.02 (M^+, 28), 327.00 (28), 242.98 (100).

4.3.19. *N*′-2-Propylidene-1-(3-nitrophenyl)-β-carboline-3-carbohydrazide (45)

Yield: 85%; mp >300 °C; IR (KBr) ν_{max}: 1623 (C=N), 1674 (C=O), 1561–1345 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.05 (s, 3H, H_3-3″), 2.07 (s, 3H, H_3-2″), 7.36 (t, *J* = 7.5 Hz, 1H, H-6), 7.63 (d, *J* = 7.5 Hz, 1H, H-8), 7.70 (t, *J* = 7.5 Hz, 1H, H-7), 7.95 (t, *J* = 8.0 Hz, 1H, H-5′), 8.41 (dd, *J* = 8.0 Hz, *J* = 2.3 Hz, 1H, H-4′), 8.50 (d, *J* = 8.0 Hz, 1H, H-6′), 8.62 (d, *J* = 7.5 Hz, 1H, H-5), 8.95 (t, *J* = 1.8 Hz, 1H, H-2′), 9.00 (s, 1H, H-4), 10.97 (s, 1H, NH), 12.16 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 16.5 (CH_3, C-3″), 25.1 (CH_3, C-2″), 112.6 (CH, C-8), 114.3 (CH, C-4), 120.1 (CH, C-6), 121.1 (C, C-4b), 122.4 (CH, C-6′), 123.4 (CH, C-4′), 123.6 (CH, C-2′), 129.1 (CH, C-7), 130.7 (CH, C-5′), 134.4 (C, C-4a), 134.9 (CH, C-5), 137.8 (C, C-1′), 138.7 (C, C-9a), 139.1 (C, C-3), 141.6 (C, C-8a), 148.3 (C, C-1), 156.2 (C, C-3′), 159.6 (C=N), 176.5 (C=O); MS *m/z* (%): 387.01 (M^+, 52), 372.00 (50), 287.98 (70), 241.97 (100).

4.3.20. *N*′-2-Propylidene-1-(3-hydroxy-4-methoxyphenyl)-β-carboline-3-carbohydrazide (46)

Yield: 81%; mp >300 °C; IR (KBr) ν_{max}: 3327 (N-H), 1624 (C=N), 1675 (C=O), 1524–1347 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.06 (s, 3H, H_3-2″), 2.06 (s, 3H, H_3-3″), 3.92 (s, 3H, OCH_3), 7.03 (d, *J* = 7.8 Hz, 1-H, H-5′), 7.32 (t, *J* = 7.8 Hz, 1H, H-6), 7.57–7.62 (m, 2H, H-7 and H-8), 7.70 (d, *J* = 7.8 Hz, 1H, H-6′), 7.74 (d, *J* = 1.8 Hz, 1 H, H-2′), 8.43 (d, *J* = 7.8 Hz, 1H, H-5), 8.85 (s, 1H, H-4), 9.49 (s, 1H, NH), 11.03 (s, 1H, OH), 11.88 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 16.3 (CH_3, C-3″), 25.1 (CH_3, C-2″), 55.5 (OCH_3), 112.2 (CH, C-8), 112.6 (CH, C-4), 112.7 (CH, C-2′), 115.5 (CH, C-5′), 120.2 (CH, C-6), 121.2 (C, C-4a), 121.2 (CH, C-6′), 122.0 (CH, C-5), 128.5 (CH, C-7), 129.7 (C, C-1′), 134.0 (C, C-9a), 138.6 (C, C-3), 140.9 (C, C-8a), 141.4 (C, C-1), 147.9 (C, C-4′), 148.1 (C, C-3′), 154.9 (C=N), 159.9 (C=O); MS *m/z* (%): 388.03 (M^+, 90), 373.00 (95), 28.99 (100), 256.95 (87).

4.3.21. *N*′-2-Propylidene-1-(4-hydroxyphenyl)-β-carboline-3-carbohydrazide (47)

Yield: 76%; mp >300 °C; IR (KBr) ν_{max}: 3315 (N-H), 1666 (C=O), 1561–1381 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.03 (s, 3H, H_3-3″), 2.06 (s, 3H, H_3-2″), 7.03 (d, *J* = 8.4 Hz, 2H, H-3′ and H-5′), 7.32 (t, *J* = 7.5 Hz, 1H, H-6), 7.60 (t, *J* = 7.5 Hz, 1H, H-7), 7.70 (d, *J* = 7.5 Hz, 1H, H-8), 8.00 (d, *J* = 8.4 Hz, 2H, H-2′ and H-6′), 8.43 (d, *J* = 7.5 Hz, 1H, H-5), 8.84 (s, 1H, H-4), 9.90 (s, 1H, NH), 10.98 (s, 1H, OH), 11.85 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 16.4 (CH_3, C-3″), 25.0 (CH_3, C-2″), 112.6 (CH, C-4), 112.7 (CH, C-8), 115.7 (2CH, C-3′ and C-5′), 120.2 (CH, C-6), 121.2 (C, C-4a), 122.0 (C, C-5), 128.2 (C, C-4b), 128.5 (CH, C-7), 129.7 (C, C-3), 129.9 (2CH, C-2′ and C-6′), 134.9 (C, C-1′), 138.6 (C, C-9a), 140.9 (C, C-8a), 141.4 (C, C-1), 155.2 (C, C-4′), 158.5 (C=N), 159.9 (C=O); MS *m/z* (%): 358.04 (M^+, 32), 343.03 (30), 258.99 (100).

4.3.22. *N*′-2-Propylidene-1-[4-(dimethylamino)phenyl]-β-carboline-3-carbohydrazide (48)

Yield: 82%; mp 272–274 °C; IR (KBr) ν_{max}: 1671 (C=O), 1555–1347 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.05 (s, 3H, H_3-3″), 2.16 (s, 3H, H_3-2″), 3.05 (s, 6H, $N(Me)_2$), 6.90 (d, *J* = 8.7 Hz, 2H, H-3′ and H-5′), 7.29–7.38 (m, 1H, H-6), 7.56–7.58 (m, 2H, H-7 and H-8), 7.89 (d, *J* = 8.7 Hz, 2H, H-2′ and H-6′), 8.16 (d, *J* = 7.8 Hz, 1H, H-5), 8.87 (s, 1H, H-4), 8.95 (s, 1H, NH), 11.08 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 16.7 (CH_3, C-3″), 25.7 (CH_3, C-2″), 40.5 $(CH_3)_2$, 111.9 (CH, C-8), 112.7 (2CH, C-3′ and C-5′), 113.3 (CH, C-4), 121.0 (CH, C-6), 122.3 (CH, C-5), 122.5 (C, C-4b), 125.6 (C-4a), 128.7 (CH, C-7), 129.0 (2CH, C-2′ and C-6′), 130.3(C, C-1′), 134.8 (C-9a), 139.7 (C, C-3), 140.7 (C, C-8a), 141.6 (C, C-1), 151.1 (C, C-4′), 154.5 (C=N), 161.4 (C=O); MS *m/z* (%): 385.07 (M^+, 85), 370.05 (77), 286.03 (100), 241.98 (72).

4.3.23. *N*′-2-Propylidene-1-(4-nitrophenyl)-β-carboline-3-carbohydrazide (49)

Yield: 85%; mp >300 °C; IR (KBr) ν_{max}: 3249 (N-H), 1626 (C=N), 1672 (C=O), 1515–1341 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6/$CDCl_3$ 3:1): δ 2.03 (s, 3H, H_3-3″), 2.07 (s, 3H, H_3-2″), 7.36 (t, *J* = 7.5 Hz, 1H, H-6), 7.65 (t, *J* = 7.5 Hz, 1H, H-7), 7.71 (d, *J* = 7.5 Hz, 1H, H-8), 8.43–8.52 (m, 5H, H-2′, H-3′, H-5′, H-6′ and H-5), 9.02 (s, 1H, H-4), 10.92 (s, 1H, NH), 12.48 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 16.4 (CH_3, C-3″), 25.0 (CH_3, C-2″), 112.4 (CH, C-8), 114.3 (CH, C-4), 120.3 (CH, C-6), 120.9 (C, C-4a), 121.9 (CH, C-5), 123.6 (2CH, C-3′ and C-5′), 128.8 (CH, C-7), 129.6 (2CH, C-2′ and C-6′), 130.7 (C, C-1′), 134.6 (C, C-3), 138.9 (C, C-9a), 141.6 (C, C-8a), 143.5 (C, C-1), 147.3 (C, C-4′), 155.5 (C=N), 159.7 (C=O); MS *m/z* (%): 387.02 (M^+, 55), 371.99 (48), 287.96 (75), 241.97 (100).

4.3.24. *N*′-2-Propylidene-1-(2-chlorophenyl)-β-carboline-3-carbohydrazide (50)

Yield: 75%; mp 233–236 °C; IR (KBr) ν_{max}: 1624 (C=N), 1682 (C=O), 1595–1345 (C=C) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.93 (s, 3H, H_3-3″), 2.03 (s, 3H, H_3-2″), 7.30–7.36 (m, 1H, H-6), 7.57–7.65 (m, 4H, H-5′, H-6′, H-7 and H-8), 7.73–7.76 (m, 1H, H-3′), 7.79–7.82 (m, 1H, H-4′), 8.47 (d, *J* = 7.8 Hz, 1H, H-5), 8.99 (s, 1H, H-4), 10.79 (s, 1H, NH), 11.81 (s, 1H, 9-NH); ^{13}C NMR (75.5 MHz): δ 16.4 (CH_3, C-3″), 25.0 (CH_3, C-2″), 112.4 (CH, C-8), 114.1 (CH, C-4), 120.3 (CH, C-6), 121.0 (C, C-4b), 122.3 (CH, C-5), 127.6 (CH, C-7), 128.9 (CH, C-5′), 129.4 (C, C-2′), 129.4 (C, C-4a), 130.2 (C, C-3′), 130.7 (CH, C-6′), 132.4 (CH, C-4′), 135.2 (C, C-1′), 135.8 (C,C-9a), 138.4 (C, C-3), 139.3 (C, C-8a), 141.4 (C, C-1), 155.3 (C═N), 159.8 (C═O); MS *m/z* (%): 376.01 (M^+, 45), 361.00 (48), 276.97 (85), 241.99 (100).

4.4. General procedure for the synthesis of *N*′-(substituted-benzylidene)-1-(substituted-phenyl)-9-methyl-β-carboline-3-carbohydrazides (53–56)

To a solution of the methyl esters **1a–c** (1.9 mmol) in anhydrous DMF (15 mL) were added NaOH (0.304 mg,7.6 mmol) and methyl iodide (0.118 mL, 19 mmol) at room temperature. The mixture was stirred for 48 h, diluted with H_2O (250 mL) and extracted with EtOAc. The organic layer was dried over N_2SO_4, filtered and evaporated. The solid residues obtained were recrystallized from methanol to afford the products **51a–c**. The N^9-methylated derivatives **51a–c** (4.0 mmol) in EtOH (50 mL) were treated with hydrazine hydrate (53 mmol) and the mixture was refluxed for 72 h and then cooled to 0 °C. The formed solids were collected by filtration and washed with EtOH, to furnish the corresponding carbohydrazides **52a–c.**

A solution of the carbohydrazides **52a–c** (1.0 mmol) in H_2O (10 mL) and two drops of concentrated H_2SO_4 was heated to 65 °C, and the respective aldehydes, dissolved in EtOH (10 mL), were added. The resulting solution was refluxed for 48 h. After cooling, the mixture was poured onto ice-water, neutralized with 10% aqueous Na_2CO_3 and the formed solids were collected by filtration and washed with water.

4.4.1. *N*′-(4-Dimethylaminobenzylidene)-9-methyl-1-(3-nitrophenyl)-β-carboline-3-carbohydrazide (53)

Yield: 75%; mp 232.0–234.0 °C; IR (KBr) ν_{max}: 1671 (C═O), 1525–1348 (C═C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.03 (s, 6H, $N(CH_3)_2$), 3.55 (s, 3H, N-CH_3), 6.71 (d, *J* = 8.7 Hz, 2H, H-3″ and H-5″), 7.43 (t, *J* = 8.1 Hz, 1H, H-7), 7.50 (d, *J* = 8.1 Hz, 1H, H-8), 7.71 (d, *J* = 8.7 Hz, 2H, H-2″ and H-6″), 7.80 (t, *J* = 8.1 Hz, 1H, H-6), 8.15 (s, 1H, N═CH), 8.31 (d, *J* = 8.1 Hz, 1H, H-5), 8.42-8.45 (m, 2H, H-5′ and H-6′), 8.58 (t, *J* = 1.8 Hz, 1H, H-2′), 9.11 (s, 1H, H-4), 10.8 (s, 1H, NH); ^{13}C NMR (75.5 MHz): δ 33.7 (N–CH_3), 40.4 $(CH_3)_2$, 110.3 (CH, C-8), 111.8 (2CH, C-3″ and C-5″), 115.0 (CH, C-4), 121.5 (C, C-4b), 121.5 (CH, C-6), 121.5 (CH, C-7), 122.4 (CH,C-5), 123.8 (CH, C-5′), 124.9 (CH, C-2′), 129.5 (2CH, C-2″ and C-6″), 129.6 (C, C-1″), 129.7 (C, C-1′), 131.9 (CH, C-6′), 135.9 (CH,C-4′), 136.5 (C, C-3), 139.4 (C, C-9a), 141.2 (C, C-8a), 143.6 (C, C-1), 148.3 (C, C-3′), 149.3 (N═CH), 152.0 (C, C-4″), 160.6 (C═O); MS *m/z* (%): 492.09 (M^+, 40), 346.01 (40), 303.00 (75), 256.1 (57), 145.99 (100).

4.4.2. *N*′-Phenylbenzylidene-1-(4-methoxyphenyl)-9-methyl-β-carboline-3-carbohydrazide (54)

Yield: 83%; mp 216.0–219.0 °C; IR (KBr) ν_{max}: 1686 (C═O), 1610 (C═N), 1511–1358 (C═C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.54 (s, 3H, N-CH_3), 3.95 (s, 3H, O-CH_3), 7.12 (d, *J* = 8.4 Hz, 2H, H-3′ and H-5′), 7.35–7.47 (m, 4H, H-6, 7, 8 and H-4″), 7.59–7.66 (m, 4H, H-2′, 6′, H-2″ and H-6″), 7.79–7.82 (m, 2H, H-3″ and H-5″), 8.25 (s, 1H, N═CH), 8.27 (s, 1H, H-5), 9.01 (s, 1H, H-4), 11.14 (s, 1H NH); ^{13}C NMR (75.5 MHz): δ 33.2 (N–CH_3), 55.6 (O-CH_3), 110.2 (CH, C-8), 113.9 (2CH,C-3′ and C-5′), 114.2 (CH, C-4), 121.0 (CH, C-6), 121.7 (C, C-4b), 122.2 (CH,C-5), 127.9 (2CH, C-3″ and C-5″), 128.8 (2CH, C-2″ and C-6″), 129.1 (CH, C-7), 130.4 (CH, C-4″), 130.9 (C, C-4a), 131.1 (2CH,C-2′ and C-6′), 131.8 (C, C-1′), 134.1 (C, C-1″), 136.8 (C, C-9a), 138.4 (C, C-3), 142.4 (C, C-8a), 143.4 (C, C-1), 148.0 (N═C), 160.3 (C, C-4′), 161.5 (C═O); MS *m/z* (%): 434.07 (M^+, 12), 331.04 (100), 314.04 (53), 288.04 (70).

4.4.3. *N*′-(2-Chlorobenzylidene)-1-(4-methoxyphenyl)-9-methyl-β-carboline-3-carbohydrazide (55)

Yield: 78%; mp 217.0–220.0 °C; IR (KBr) ν_{max}: 1673 (C═O), 1510–1357 (C═C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.55 (s, 3H, 9-N-CH_3), 3.95 (s, 3H, OCH_3), 7.13 (d, *J* = 8.7 Hz, 2H, H-3′ and H-5′), 7.30–7.36 (m, 3H, H-6, H-5″ and H-6″), 7.40 (t, *J* = 7.5 Hz, 1H, H-4″), 7.47 (d, *J* = 7.5 Hz, 1H, H-8), 7.62 (d, *J* = 8.7 Hz, 2H, H-2′ and H-6′), 7.67 (d, *J* = 7.5 Hz, 1H, H-3″), 8.28 (d, *J* = 7.5 Hz, 1H, H-5), 8.31–8.33 (m, 1H, H-7), 8.65 (s, 1H, N═C), 9.04 (s, 1H, H-4), 11.26 (s, 1H, NH); ^{13}C NMR (75.5 MHz): δ 33.2 (N–CH_3), 55.6 (OCH_3), 110.2 (CH, C-8), 114.0 (2CH, C-3′ and C-5′), 114.4 (CH, C-4), 121.1 (CH, C-6), 121.7 (C, C-4b), 122.2 (CH, C-5), 127.2 (CH, C-6″), 128.3 (CH, C-7), 129.2 (CH, C-3″), 129.8 (CH, C-5″), 130.9 (C, C-4a), 131.1 (2CH, C-2′ and C-6′), 131.3 (C, C-4″), 131.6 (CH, C-2″), 131.7 (C, C-1″), 134.3 (C, C-1′), 136.8 (C, C-9a), 138.2 (C, C-3), 142.5 (C, C-1), 143.4 (C, C-8a), 144.2 (N═CH), 160.3 (C, C-4′), 161.6 (C═O); MS *m/z* (%): 468.01 (M^+, 0.5), 331.02 (100), 314.03 (50), 288.03 (70).

4.4.4. *N*′-(2-Chlorobenzylidene)-9-methyl-1-phenyl-β-carboline-3-carbohydrazide (56)

Yield: 82%; mp 260.0–263.0 °C; IR (KBr) ν_{max}: 1692 (C═O), 1557–1351 (C═C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.52 (s, 3H, N-CH_3), 7.31–7.49 (m, 5H, H-6, 7, 8, H-5″ and H-6″), 7.60–7.71 (m, 7H, H-2′, H-3′, H-4′, H-5′, H-6′, H-3″ and H-4″), 8.28–8.33 (m, 1H, H-5), 8.66 (s, 1H, N═CH), 9.08 (s, 1H, H-4), 11.26 (s, 1H, NH); ^{13}C NMR (75.5 MHz): δ 33.2 9 (N–CH_3), 110.2 (CH, C-8), 114.7 (CH, C-4), 121.2 (CH, C-6), 121.7 (C-4b), 122.3 (CH, C-5), 127.2 (CH, C-5″), 128.3 (CH, C-3″), 128.6 (2CH, C-3′ and C-5′), 129.2 (CH, C-4″), 129.3 (C, C-4′), 129.8 (CH, C-7), 129.9 (2CH, C-2′ and C-6′), 131.0 (C, C-4a), 131.3 (CH, C-6″), 131.6 (C, C-1′), 134.4 (C, C-1″), 136.7 (C, C-2″), 138.3 (C, C-3), 139.4 (C, C-9a), 142.6 (C, C-8a), 143.4 (C, C-1), 144.3 (C═N), 161.6 (C═O); MS *m/z* (%): 438.02 (M^+, 0.5), 301.03 (90), 284.02 (40), 258.02 (100), 257.02 (90).

4.5. Biological assays

4.5.1. Animals

Balb/C mice (20–35 g) obtained from CEMIB-Unicamp were maintained in a room with controlled temperature 25 ± 2 °C for 12 h light/dark cycle, with free access to food and water. Animal care, research and animal sacrifice protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA) and approved by the Biology Institute/UNICAMP—Ethical Committee for Animal Research.

4.5.2. In vitro anticancer assay

The anticancer activity was assessed by sulforodamine B (SRB) colorimetric assay developed by the National Cancer Institute, using doxorubicin as a positive control.[36] Assays were performed in a 96-well plate using four concentrations at 10-fold dilutions (0.25–250 mg mL^{-1}) for each tested compound. The anticancer activity was deduced from concentration–response curves. The IC_{50} value refers to the drug concentration that produces a 50% reduction in cellular growth when compared to untreated control cells.[37]

4.5.3. In vivo antineoplastic assay: Ehrlich solid carcinoma in mice paw

The most active compound **4** was tested for its in vivo in the Ehrlich solid carcinoma assay model. The procedures were developed according to Vendramini-Costa et al.,[35] with few modifications on data analysis. The Ehrlich ascitic tumor (EAT), derived from a spontaneous murine mammary adenocarcinoma, was maintained in the ascitic form by sequential passages in *Swiss* mice, by means of weekly ip transplantations of 5×10^5 tumor cells, in order to prepare cells for the following test. The ascitic fluid was removed by opening the belly and carefully collecting all the fluid using a sterile 3 mL syringe. Ascitic tumor cell counts were performed in a Neubauer hemocitometer, and the total number was determined by the Trypan blue dye exclusion method, with tumor cell viability always higher than 90%. The cells were then diluted in 0.9% phosphate buffer saline (PBS) for final inoculation density (2.5×10^6 cells/mL). For the solid form implantation, 2.5×10^6 viable tumor cells in a volume of 60 μL were injected in sub-plantar site of the right hind paw of *Balb-C* mice.[38] After tumor cell inoculation, the foot volume was measured every three days using a plethysmometer apparatus (Panlab, Spain) till the 15th day when the animals were sacrificed. The tumor growth was measured considering the following formula: Volume measured − Basal volume = Tumor volume.

4.5.4. Statistical analysis

The results were submitted to one way analysis of variance (ANOVA), considering as critical level $p \leqslant 0.05$ to evaluate significant difference between the control and treated groups, followed by Duncan's Test, using StatSoft® software. Graphs were designed using the Origin® software.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.08.059.

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