

Article

Synthesis and Evaluation of New β -Carboline-3-(4-benzylidene)-4H-oxazol-5-one Derivatives as Antitumor Agents

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Abstract: In the present work, we report the synthesis and *in vitro* anticancer and antimicrobial activity evaluation of a new series of 1-substituted- β -carboline derivatives bearing a 4-benzylidene-4H-oxazol-5-one unity at C-3. The compound 2-[1-(4-methoxyphenyl)-9H- β -carbolin-3-yl]-4-(benzylidene)-4H-oxazol-5-one (**11**) was the most active derivative, exhibiting a potent cytotoxic activity against glioma (U251), prostate (PC-3) and ovarian (OVCAR-03) cancer cell lines with IC₅₀ values of 0.48, 1.50 and 1.07 μ M, respectively. An *in silico* study of the ADME properties of the novel synthesized β -carboline derivatives was also performed.

Keywords: β -carboline; 4H-oxazol-5-one; cytotoxic activity; antimicrobial activity

1. Introduction

Natural and synthetic tetrahydro- β -carbolines and β -carbolines are a class of alkaloids with a large spectrum of important pharmacological properties [1–7]. Among the activities presented, the antitumor

activity has received special attention, and several studies on structure-activity relationship of β -carbolines have focused their anticancer activities [8–16]. The SAR studies have demonstrated that the introduction of appropriate substituents into the positions –1, –2, –3 and –9 of the β -carboline nucleus resulted in more potent antitumor β -carboline derivatives, with reduced toxicity.

The potential of β -carbolines and the importance of the search for new antitumor agents led our group to a continuing study of this class of compounds. In previous works, we reported the synthesis and *in vitro* antitumor activities, against a panel of human cancer cell lines, of a series of 1-substituted β -carboline derivatives bearing different substituents at C-3, such as 2-substituted-1,3,4-oxadiazole and 5-substituted-1,2,4-triazole rings [17], 3-alkylamino(methyl)-2-thioxo-1,3,4-oxadiazole groups [18] and *N*-(substituted-benzylidene)-carbohydrazide groups [19]. The anticancer assay results indicated several compounds with potent anticancer activity, with IC_{50} values lower than 1.0 μ M for some of the human cancer cell lines tested [17–19]. In addition to the antitumor activity, β -carbolines containing the 2-thioxo-1,3,4-oxadiazole group at C-3 exhibited antimicrobial activity towards the fungus *Candida albicans* and the bacterium *Bacillus subtilis* [18]. Antimicrobial activity of β -carboline derivatives, mainly against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* bacteria and against *Candida albicans* fungi, was also reported in literature [20,21].

With the aim of evaluating the influence of different substituents at position-3 of 1-substituted- β -carbolines, in this work we propose the incorporation of a benzylidene-4*H*-oxazol-5-one unity at C-3, expecting an improvement of the antitumor and antimicrobial activities in relation to the most active β -carboline derivatives reported in our previous work [17–19]. The 4*H*-oxazol-5-one ring is found in natural and synthetic compounds possessing important biological activities, such as antimicrobial [22–24], antiviral [25], antiangiogenic [26], inhibitory of tyrosinase [27], cytotoxic and immunomodulatory properties [28]. Due their biological importance, several methods were reported for the synthesis of oxazolones [29–31].

Thus, in the present work, we report the synthesis and *in vitro* cytotoxic and antimicrobial activity evaluation of some novel 1-substituted β -carboline derivatives bearing a 4-(benzylidene)-4*H*-oxazol-5-one moiety at C-3. Additionally, an *in silico* study of the ADME properties of the novel synthesized β -carboline derivatives was carried out by investigating their Lipinski's parameters, topological polar surface area (TPSA) and percentage of absorption (% ABS).

2. Results and Discussion

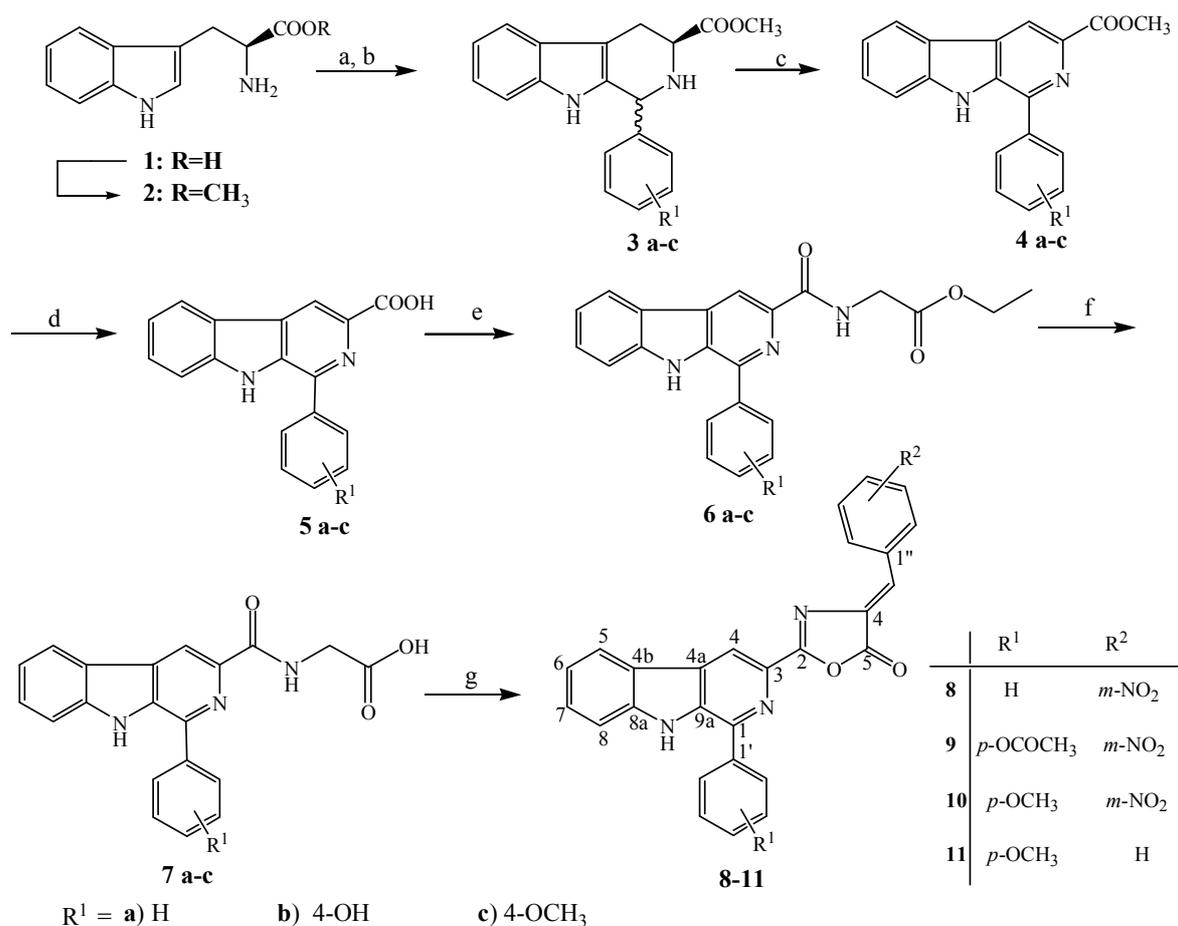
2.1. Chemistry

The synthetic route for the β -carboline-3-(4-benzylidene)-4*H*-oxazol-5-ones **8–11** is outlined in Scheme 1. The 1-substituted β -carboline-3-carboxylic acids **5a–c** were prepared from commercial L-tryptophan (**1**), according to the synthetic protocol described by our group [17–19]. The Pictet-Spengler condensation of L-tryptophan methyl ester (**2**) with benzaldehyde, 4-hydroxybenzaldehyde and 4-methoxybenzaldehyde afforded the 1,2,3,4-tetrahydro- β -carbolines **3a–c**. Oxidation of **3a–c** with sulfur in refluxing xylene, led to the methyl β -carboline-3-carboxylates **4a–c**, which were hydrolyzed under basic conditions to give the β -carboline-3-carboxylic acids **5a–c**.

In order to synthesize the β -carboline-3-(4-benzylidene)-4*H*-oxazol-5-ones **8–11**, the Erlenmeyer-Plöchl reaction, the most common route to oxazolones, was employed [31]. The derivatives **5a–c** were converted to the respective *N*-(1-benzylidene- β -carboline-3-carbonyl)-glycine ethyl esters **6a–c** by activation of the β -carboline-3-carboxylic acids **5a–c** with *N,N'*-dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP), in pyridine, followed by treatment with glycine ethyl ester hydrochloride [32]. To prepare the β -carboline-3-carbonyl-amino acids **7a–c**, the corresponding esters **6a–c** were submitted to hydrolysis with sodium carbonate in refluxing methanol/water. Erlenmeyer-Plöchl reaction of **7a** and **7c** with 3-nitrobenzaldehyde or benzaldehyde, afforded the corresponding β -carboline-3-oxazolones **8–11**. The reaction of the derivative **7b**, bearing the 4-hydroxyphenyl substituent at C-1, led to the oxazolone **9** with the hydroxyl group acetylated.

To evaluate the effects of electron-donating groups at the R² position of the benzylidene-4*H*-oxazol-5-one moiety on activity, compounds **7a** and **7c** were subjected to Erlenmeyer-Plöchl reaction with 4-*N,N*-dimethylaminobenzaldehyde and 4-methoxybenzaldehyde. However, these reactions failed to furnish the corresponding β -carboline-3-oxazolones, probably due to the relative low electrophilicity of the aldehydes employed.

Scheme 1. The synthetic route to compounds **8–11**.



Reagents and conditions: (a) CH₃OH, H₂SO₄, reflux, 48 h, 95%; (b) Aldehyde (R¹CHO), TFA, CH₂Cl₂, reflux, 48 h, 75–95%; (c) Sulfur, xylene, reflux, 48 h, 75–85%; (d) NaOH, H₂O:CH₃OH 2:1, reflux, 24 h and HCl 5 M, 0 °C, 3 h, 85–90%; (e) HCl.NH₂CH₂COOCH₂CH₃, DMAP, DCC, pyridine, CH₂Cl₂, RT, 48 h, 45–56%; (f) Na₂CO₃, H₂O:CH₃OH 2:1, reflux, 24 h and HCl 5 M, 0 °C, 3h, 68–77%; (g) Aldehyde (R²CHO), AcONa, (CH₃CO)₂O, 80 °C, 24 h, 30–42%.

All novel compounds were characterized by their spectroscopic data (IR, HR-ESIMS, ^1H and ^{13}C -NMR), which are described in the Experimental section. The (4-benzylidene)-4*H*-oxazol-5-one group at C-3 of **8–11** was evidenced by the presence of a singlet at δ_{H} 7.54–7.56 in the ^1H -NMR spectra, corresponding to the benzylidene hydrogen, which showed a correlation with the carbon signals at δ_{C} 126.4–131.5, in the HSQC spectra. The signals at δ_{C} 164.0–164.5 and 168.0–166.9, in the ^{13}C -NMR spectra, were assigned to the C-2 and C-5 carbons, respectively, of the 4*H*-oxazol-5-one ring. The IR spectra showed absorption bands characteristic for C=O stretching in the 1,780–1,816 cm^{-1} region.

The structures of **8–11** were also confirmed by their HR-ESI and EI mass spectra. The compounds showed the presence of the molecular ions $[\text{M}^+]$ consistent with the expected structures, and a base peak at m/z $[\text{M}^+$ -substituted-benzylidene oxazolone] corresponding to the cleavage between C-3 of β -carboline and C-2 of the 4*H*-oxazol-5-one ring.

2.2. Cytotoxic Activity

The IC_{50} values obtained for the synthesized compounds are shown in Table 1. Analysis of the IC_{50} values (Figure 1, Table 1) showed that compound **8** was active only against the melanoma (UACC-62) cell line, with IC_{50} of 7.52 μM . The same result was observed for the derivative **9**, where the phenyl group at C-1 was changed for a 4-acetoxyphenyl group.

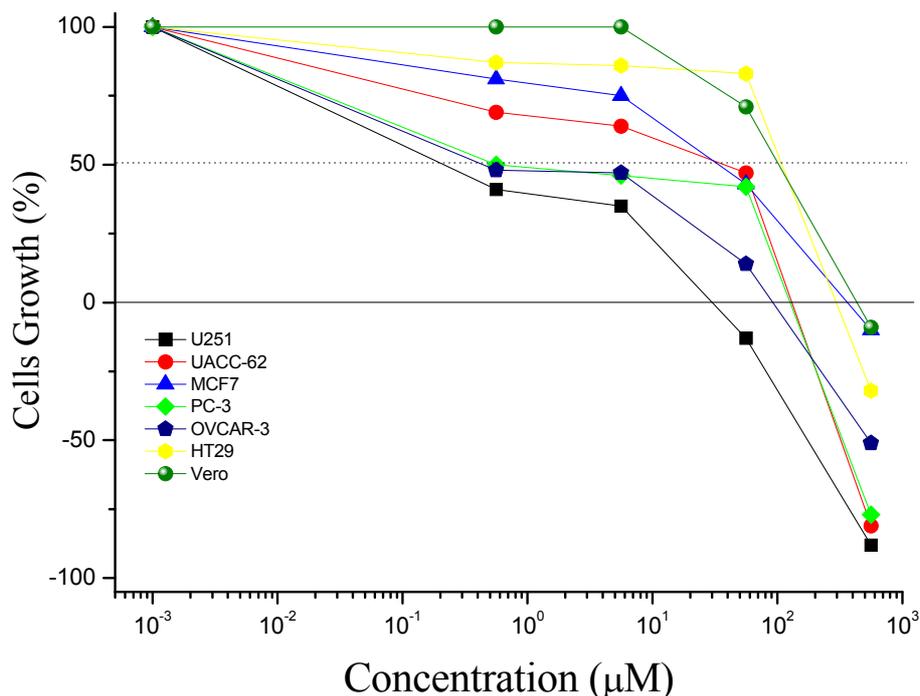
On the other hand, the substitution of the phenyl group at C-1, in compound **8**, for a 4-methoxyphenyl group resulted in the more active compound **10**, showing that a good electron-donating substituent at the phenyl ring is important for the cytotoxicity. A potent activity against glioma (U251) and ovarian (OVCAR-03) cell lines was observed for compound **10**, which presented IC_{50} values of 0.35 and 2.18 μM , respectively. Keeping the 4-methoxyphenyl group at C-1 and in order to evaluate the effect on activity of other groups at the oxazolone moiety, the 3-nitrophenyl group was substituted for a phenyl group, resulting in compound **11**, which displayed the better activity in comparison to all other synthesized compounds. Derivative **11** showed IC_{50} values ≤ 10.00 μM for four of the cells lines tested (Table 1). A potent activity was observed mainly against glioma (U251), prostate (PC-3) and ovarian (OVCAR-03) cell lines with IC_{50} values of 0.48, 1.50 and 1.07 μM , respectively.

Table 1. IC_{50} values (in μM) for compounds **8–11**.

	Glioma U251	Melanoma UACC-62	Breast MCF7	Prostate PC-3	Ovarian OVCAR-03	Colon HT-29	VERO
Doxorubicin ^a	0.03	0.04	0.06	0.05	0.42	0.18	0.50
8	62.42	7.52	>100	>100	63.19	>100	>100
9	80.20	8.76	>100	>100	>100	>100	>100
10	0.35	15.93	47.63	5.28	2.18	>100	>100
11	0.48	10.00	23.44	1.50	1.07	67.88	63.17

^a Doxorubicin was the positive control.

In addition to the effective growth inhibition (IC_{50} values), compound **11** showed also cytostatic activity, with IC_{100} values of 15.50, 56.83, 64.17 and 112.42 μM for glioma (U251), ovarian (OVCAR-03), prostate (PC-3) and melanoma (UACC-62) human cell lines, respectively (Figure 1).

Figure 1. Concentration (μM) versus cell growth (%) for compound **11**.

The comparison of IC_{50} data of **11** (Table 1) with those of the most active β -carboline derivatives reported in our previous work [17–19] demonstrated, in general, a lower cytotoxic activity for this compound. However, specifically for the prostate (PC-3) and ovarian (OVCAR-03) cancer cell lines, the cytotoxic activity of **11** was similar to that of some active reported compounds, such as 1-(4-*N,N*-dimethylaminophenyl)-3-(5-thioxo-1,2,4-triazol-3-yl)- β -carboline (prostate: $\text{IC}_{50} = 1.37 \mu\text{M}$; ovarian: $\text{IC}_{50} = 1.09 \mu\text{M}$) [17] and *N'*-(2-chlorobenzylidene)-1-(4-hydroxyphenyl)- β -carboline-3-carbohydrazide (prostate: $\text{IC}_{50} = 1.83 \mu\text{M}$, ovarian: $1.65 \mu\text{M}$) [19].

The IC_{50} data shown in Table 1 were also compared with those of previously reported β -carboline derivatives bearing the 4-methoxyphenyl group at position-1 and the 2-thioxo-1,3,4-oxadiazole, 2-methylthio-1,3,4-oxadiazole and 5-thioxo-1,2,4-triazole groups [17], and (substituted benzylidene)-carbohydrazide groups [19], at position-3. This comparison pointed out that, except for 1-(4-methoxyphenyl)-3-(2-methylthio-1,3,4-oxadiazol-5-yl) β -carboline (ovarian: $\text{IC}_{50} = 2.13 \mu\text{M}$) [17], the synthesized oxazoles **10** and **11** showed higher cytotoxic activity, particularly for prostate (PC-3) and ovarian (OVCAR-03) cancer cell lines, than all other 1-(4-methoxyphenyl)-3-substituted- β -carboline derivatives previously tested [17,19]. These data demonstrate the importance of the (substituted benzylidene)-4*H*-oxazol-5-one moiety on the cytotoxic activity of compounds **10** and **11**.

2.3. Antimicrobial Activity

Compounds **8–11** were assayed against the bacteria *Bacillus subtilis* ATCC 2576, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 13388, *Staphylococcus aureus* ATCC 6538, and against the fungi *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC-22019 and *Candida tropicalis* ATCC-28707. Contrarily to the expectation, the assay results showed no activity for compounds **8–11** ($\text{IC}_{50} > 100 \mu\text{M}$), indicating that the (substituted-benzylidene)-4*H*-oxazol-5-one group at C-3 did not contribute to the antimicrobial activity.

2.4. In Silico Study

An *in silico* computational study of the synthesized β -carboline-3-(4-benzylidene)-4*H*-oxazol-5-ones **8–11** was performed by determining the Lipinski's parameters, topological polar surface area (TPSA) and percentage of absorption (% ABS) [33–35]. Calculations were performed using the *Molinspiration Online Property Calculation Toolkit* (www.molinspiration.com) [34] and *OSIRIS Property Explorer* (www.organic-chemistry.org/prog/peo) [35]. The percentage of absorption was estimated using the equation: % ABS = 109 – 0.345 × TPSA [33]. These data are shown in Table 2.

In vivo absorption of the new synthesized derivatives was tentatively assessed by means of theoretical calculations following Lipinski's rule of five, which establishes that the absorption or permeation of an orally administered compound is more likely to be good if the drug satisfies the following criteria: (a) hydrogen bond donors ≤ 5 (OH and NH groups); (b) hydrogen bond acceptors ≤ 10 (N and O atoms); (c) molecular weight < 500; (d) calculated logP < 5 [33–35]. Compounds violating more than one of these rules may present bioavailability problems.

Table 2. Lipinski's parameters and % ABS, TPSA, LogS for compounds **8–11**.

Comp.	%ABS	TPSA ^a (Å ²)	Lipinski's parameters					
			nHBA (NO)	nHBD (OHNH)	logP ^a	MW	n violations	logS ^a
8	68.42	117.61	8	1	5.45	460.45	1	−7.41
9	59.35	143.91	10	1	5.00	518.48	1	−7.71
10	65.24	126.85	9	1	5.50	490.47	1	−7.43
11	81.05	81.02	6	1	5.57	445.48	1	−6.97

^a www.molinspiration.com; ^b www.organic-chemistry.org/prog/peo; %ABS = 109 – 0.345 × TPSA; Number hydrogen bond acceptor (NO) = nHBA ≤ 10 ; Number hydrogen bond donors (OHNH) = nHBD ≤ 5 ; MW ≤ 500 ; Octanol-water partition coefficient = LogP < 5; Solubility = LogS > −4.

Our results (Table 2) revealed that the β -carboline-3-(4-benzylidene)-4*H*-oxazol-5-ones **8–11** violated only one of the Lipinski's rules. Compound **9** has a molecular weight larger than 500 g/mol and the derivatives **8**, **10** and **11** showed octanol-water partition coefficients (LogP) larger than 5.0.

All derivatives have number of hydrogen bond acceptors (*n*-ON = 6–10) and donors (*n*-OHNH = 1) in agreement with Lipinski's rule. The calculated percent absorption (% ABS) of all derivatives ranged between 59.35 and 81.05%, indicating that these compounds should have good cellular plasmatic membrane permeability.

3. Experimental

3.1. Chemistry

Melting points were determined in a Micro-Química MQAPF-301 apparatus and are uncorrected. ¹H- and ¹³C-NMR spectra were recorded in a Varian model Mercury plus 300 spectrometer (Palo Alto, CA, USA) at 300 MHz and 75.5 MHz, respectively, with DMSO-*d*₆ as solvent and TMS as the internal standard. EI-MS spectra were recorded in a Thermoelectron Corporation Focus-DSQ II spectrometer (Austin, TX, USA). HR-ESI mass spectra were recorded on a Q-TOF (Micromass) spectrometer in

positive ion mode (Manchester, UK). IR spectra were recorded on a BOMEM model MB-100 spectrometer (Quebec, CA, USA). For TLC, Merck precoated plates (silica gel 60 G254) were used. Silica gel 60 Merck (230–400 mesh) was used in the column chromatography purification of some compounds. All reagents were purchased from commercial suppliers.

3.2. General Method for Synthesis of *N*-(1-benzylidene- β -carboline-3-carbonyl)-glycine Ethyl Esters **6a–c**

To a solution of β -carboline-3-carboxylic acids **5a–c** (0.7 mmol) in pyridine (5 mL) at 0 °C was added glycine ethyl ester hydrochloride (0.7 mmol), DMAP (0.07 mmol) in CH₂Cl₂ (5 mL). The mixture was stirred for 5 min and a solution of DCC (0.7 mmol) in CH₂Cl₂ (5 mL) was added, followed by stirring for 2 h, at 0 °C, and for 24 h at room temperature. This step was repeated with a new amount of DCC (0.7 mmol) in CH₂Cl₂ (5 mL), followed by evaporation of the solvent, addition of CH₂Cl₂ and filtration of the DCU precipitate formed. The solvent was removed from the filtrate and the residual DCU separated by precipitation with acetone and filtration. The solvent was removed and the residue obtained purified on a chromatographic column (silica gel, CHCl₃/CH₃OH 5%) to give the pure β -carbolines **6a–c** in yields ranging from 45–56%.

N-(1-Phenyl- β -carboline-3-carbonyl)-glycine ethyl ester (**6a**). Yield: 55%, m.p. 194–195 °C. IR (KBr) ν_{\max} cm⁻¹: 1737 (C=O); 1657 (C=O), 1622 (C=N); 1591–1494 (C=C). ¹H-NMR (CDCl₃): δ 8.80 (s, H-4), 8.18 (d, J = 7.8 Hz, H-5), 7.35 (td, J = 7.3 Hz and 1.5 Hz, H-6), 7.49–7.63 (m, H-7), 7.49–7.63 (m, H-8), 8.89 (s, NH-9), 7.99 (dd, J = 6.9 Hz and 1.5 Hz, H-2'), 7.51–7.64 (m, H-3'/5'), 7.49–7.64 (m, H-4'), 7.99 (dd, J = 6.9 Hz and 1.5 Hz, H-6'), 8.65 (t, J = 5.7 Hz, NH-2''), 4.31 (d, J = 5.7 Hz, NHCH₂), 4.27 (q, J = 7.2 Hz, OCH₂CH₃), 1.33 (t, J = 7.2 Hz, CH₃CH₂O). ¹³C-NMR (CDCl₃): δ 141.2 (C-1), 139.8 (C-3), 113.7 (C-4), 130.6 (C-4a), 122.4 (C-4b), 122.3 (C-5), 121.1 (C-6), 129.1 (C-7), 112.1 (C-8), 140.9 (C-8a), 137.9 (C-9a), 135.0 (C-1'), 128.4 (C-2'), 129.3 (C-3'/5'), 129.3 (C-4'), 128.4 (C-6'), 166.0 (C=O), 41.7 (NHCH₂), 170.5 (C=O), 61.7 (OCH₂CH₃), 14.4 (CH₃CH₂O). EIMS, 70 eV, m/z (rel. int. %): 373 (40, M⁺), 243 (100), 271 (40).

N-[1-(4-Hydroxyphenyl)- β -carboline-3-carbonyl]-glycine ethyl ester (**6b**). Yield: 45%, m.p. 168–170 °C. IR (KBr) ν_{\max} cm⁻¹: 1729 (C=O), 1626 (C=N), 1538–1464 (C=C). ¹H-NMR (CDCl₃): δ 8.72 (s, H-4), 8.27 (d, J = 7.8 Hz, H-5), 7.28 (t, J = 7.8 Hz, H-6), 7.54 (t, J = 7.8 Hz, H-7), 7.68 (d, J = 7.8 Hz, H-8), 11.69 (s, NH-9), 8.01 (d, J = 8.7 Hz, H-2'/6'), 7.04 (d, J = 8.7 Hz, H-3'/5'), 9.71 (brs, OH), 8.97 (t, J = 6.0 Hz, NH-2''), 4.17 (d, J = 6.0 Hz, NHCH₂), 4.18 (q, J = 7.2 Hz, OCH₂CH₃), 1.27 (t, J = 7.2 Hz, CH₃CH₂O). ¹³C-NMR (CDCl₃): δ 141.3 (C-1), 138.6 (C-3), 111.9 (C-4), 127.9 (C-4a), 121.1 (C-4b), 121.3 (C-5), 119.7 (C-6), 128.2 (C-7), 112.4 (C-8), 141.0 (C-8a), 133.9 (C-9a), 129.3 (C-1'), 129.8 (C-2'/6'), 115.3 (C-3'/5'), 158.2 (C-4'), 165.2 (C=O), 40.9 (NHCH₂), 169.8 (C=O), 60.3 (OCH₂CH₃), 13.9 (CH₃CH₂O). EIMS, 70 eV, m/z (rel. int. %): 389 (25, M⁺), 259 (100), 343 (10).

N-[1-(4-Methoxyphenyl)- β -carboline-3-carbonyl]-glycine ethyl ester (**6c**). Yield: 56%, m.p. 184–186 °C. IR (KBr) ν_{\max} cm⁻¹: 1742 (C=O); 1656 (C=O), 1609 (C=N), 1561–1464 (C=C). ¹H-NMR (CDCl₃): δ 8.79 (s, H-4), 8.19 (d, J = 7.8 Hz, H-5), 7.35 (td, J = 7.0 Hz; J = 1.5 Hz, H-6), 7.53–7.61 (m, H-7), 7.53–7.61 (m, H-8), 8.81 (s, NH-9), 7.95 (dd, J = 8.7 Hz and 1.8 Hz, H-2'/6'), 7.13 (dd, J = 8.7 Hz and

1.8 Hz, H-3'/5'), 3.92 (s, OCH₃), 8.67 (t, $J = 5.5$ Hz, NH-2"), 4.32 (d, $J = 5.5$ Hz, NHCH₂), 4.27 (q, $J = 7.0$ Hz, OCH₂CH₃), 1.33 (t, $J = 7.0$ Hz, CH₃CH₂O). ¹³C-NMR (CDCl₃): δ 141.2 (C-1), 139.8 (C-3), 113.3 (C-4), 122.5 (C-4b), 122.3 (C-5), 121.1 (C-6), 128.9 (C-7), 112.0 (C-8), 140.8 (C-8a), 134.9 (C-9a), 130.5 (C-1'), 129.7 (C-2'/6'), 114.8 (C-3'/5'), 160.6 (C-4'), 55.6 (OCH₃), 166.1 (C=O), 170.5 (C=O), 41.7 (NHCH₂), 61.7 (OCH₂CH₃), 14.4 (CH₃CH₂O). EIMS, 70 eV, m/z (rel. int. %): 403 (60, M⁺), 301 (45), 273 (100), 258 (30).

3.3. General Method for the Synthesis of *N*-(1-substituted- β -carboline-3-carbonyl)-glycine **7a–c**

A suspension of *N*-(1-substituted- β -carboline-3-carbonyl)-glycine ethyl esters **6a–c** (0.5 mmol) and Na₂CO₃ (1.5 mmol) in H₂O:MeOH 2:1 (5 mL) was stirred for 24 h, at 80 °C. The mixture was cooled at 0 °C, and after stirring for 2 h, the solution was neutralized with a solution of 5 M HCl solution. The product was collected by filtration, dried and crystallized from ethanol, furnishing the compounds **7a–c** in 68–77% yield.

N-(1-Phenyl- β -carboline-3-carbonyl)-glycine (**7a**). Yield: 77%, m.p. 236–238 °C. IR (KBr) ν_{\max} cm⁻¹: 1731 (C=O); 1636 (C=N), 1593–1464 (C=C). ¹H-NMR (DMSO-*d*₆): δ 8.83 (s, H-4), 8.43 (d, $J = 7.8$ Hz, H-5), 7.32 (t, $J = 7.8$ Hz, H-6), 7.55–7.71 (m, H-7), 7.55–7.71 (m, H-8), 11.87 (s, NH-9), 8.14 (dd, $J = 7.8$ Hz and 1.2 Hz, H-2'/6'), 7.55–7.71 (m, H-3'/5'), 7.55–7.71 (m, H-4'), 8.87 (t, $J = 5.1$ Hz, NH-2"), 3.89 (d, $J = 5.1$ Hz, NHCH₂). ¹³C-NMR (DMSO-*d*₆): δ 141.6 (C-1), 139.5 (C-3), 112.9 (C-4), 129.9 (C-4a), 122.0 (C-4b), 121.2 (C-5), 120.2 (C-6), 128.9 (C-7), 112.7 (C-8), 140.6 (C-8a), 137.5 (C-9a), 134.2 (C-1'), 128.6 (C-2'/6'), 128.8 (C-3'/5'), 128.8 (C-4'), 164.7 (C=O), 41.9 (NHCH₂), 171.4 (C=O). EIMS, 70 eV, m/z (rel. int. %): 345 (20, M⁺), 301 (20), 243 (95), 60 (100).

N-[1-(4-Hydroxyphenyl)- β -carboline-3-carbonyl]-glycine (**7b**). Yield: 74%, mp decomp. IR (KBr) ν_{\max} cm⁻¹: 1729 (C=O), 1609 (C=N), 1540–1498 (C=C). ¹H-NMR (DMSO-*d*₆): δ 8.76 (s, H-4), 8.40 (d, $J = 7.6$ Hz, H-5), 7.31 (t, $J = 7.6$ Hz, H-6), 7.59 (t, $J = 7.6$ Hz, H-7), 7.70 (d, $J = 7.6$ Hz, H-8), 12.64 (s, NH-9), 8.04 (d, $J = 8.7$ Hz, H-2'/6'), 7.04 (d, $J = 8.7$ Hz, H-3'/5'), 9.86 (brs, OH), 8.95 (t, $J = 6.0$ Hz, NH-2"), 4.08 (d, $J = 6.0$ Hz, NHCH₂), 11.76 (s, OH). ¹³C-NMR (DMSO-*d*₆): δ 141.5 (C₀-1), 139.2 (C-3), 112.3 (C-4), 128.4 (C-4a), 121.3 (C-4b), 121.9 (C-5), 120.1 (C-6), 128.3 (C-7), 112.7 (C-8), 141.0 (C-8a), 133.9 (C-9a), 129.6 (C-1'), 130.1 (C-2'/6'), 115.6 (C-3'/5'), 158.4 (C-4'), 165.2 (C=O), 41.2 (NHCH₂), 171.6 (C=O). C₂₀H₁₅N₃O₄ EIMS, 70 eV, m/z (rel. int. %): 361 (20, M⁺), 260 (100), 229 (20), 60 (10).

N-[1-(4-Methoxyphenyl)- β -carboline-3-carbonyl]-glycine (**7c**). Yield: 68%, m.p. 247–249 °C. IR (KBr) ν_{\max} cm⁻¹: 1741 (C=O), 1638 (C=N), 1545–1464 (C=C). ¹H-NMR (DMSO-*d*₆): δ 8.76 (s, H-4), 8.28 (d, $J = 7.5$ Hz, H-5), 7.30 (t, $J = 7.5$ Hz, H-6), 7.56 (t, $J = 7.5$ Hz, H-7), 7.69 (d, $J = 7.5$ Hz, H-8), 11.74 (s, NH-9), 8.11 (d, $J = 8.7$ Hz, H-2'/6'), 7.91 (d, $J = 8.7$ Hz, H-3'/5'), 3.91 (s, OCH₃), 8.90 (t, $J = 5.7$ Hz, NH-2"), 4.13 (d, $J = 5.7$ Hz, NHCH₂). ¹³C-NMR (DMSO-*d*₆): δ 141.5 (C-1), 138.6 (C-3), 112.3 (C-4), 121.1 (C-4b), 121.4 (C-5), 119.9 (C-6), 128.1 (C-7), 112.5 (C-8), 140.4 (C-8a), 134.0 (C-9a), 129.5 (C-1'), 129.9 (C-2'/6'), 113.8 (C-3'/5'), 159.8 (C-4'), 55.0 (OCH₃), 164.8 (C=O), 40.9 (NHCH₂), 171.2 (C=O). EIMS, 70 eV, m/z (rel. int. %): 375 (25, M⁺), 274 (100), 229 (25), 60 (40).

3.4. General Method for Synthesis of 1-Substituted β -carboline-3-(4-benzylidene)-4H-oxazol-5-ones 8–11

To a solution of derivatives **7a–c** (0.3 mmol), in acetic anhydride (5 mL), was added 4-nitrobenzaldehyde or benzaldehyde (0.75 mmol), followed by addition of sodium acetate (1.5 mmol). The solution was stirred for 24 h at 80 °C and then, for 1h at 0 °C. The solid formed was filtered under vacuum and washed with cold acetone to afford the compounds **8–11**.

2-[1-Phenyl-9H- β -carbolin-3-yl]-4-(3-nitrobenzylidene)-4H-oxazol-5-one (8). Yield: 42%, m.p. 271–273 °C. IR (KBr) ν_{\max} cm^{-1} : 3395 (N–H), 1810 (C=O); 1625 (C=N), 1547–1497 (C=C). $^1\text{H-NMR}$ (DMSO- d_6): δ 9.17 (s, H-4), 8.49 (d, $J = 7.8$ Hz, H-5), 7.41 (t, $J = 7.8$ Hz, H-6), 7.59–7.66 (m, H-7), 7.75 (d, $J = 7.8$ Hz, H-8), 12.17 (s, NH-9), 8.12 (d, $J = 8.0$ Hz, H-2'/6'), 7.68 (d, $J = 8.0$ Hz, H-3'/5'), 7.59–7.66 (m, H-4'), 7.54 (s, C=CH), 9.30 (s, H-2''), 8.34 (d, $J = 7.5$ Hz, H-4''), 7.85 (t, $J = 7.5$ Hz, H-5''), 8.80 (d, $J = 7.5$ Hz, H-6''). $^{13}\text{C-NMR}$ (DMSO- d_6): δ 143.1 (C-1), 137.2 (C-3), 116.9 (C-4), 129.1 (C-4a), 120.9 (C-4b), 122 (C-5), 120.8 (C-6), 129.3 (C-7), 113.0 (C-8), 141.5 (C-8a), 134.8 (C-9a), 132.3 (C-1'), 128.6 (C-2'/6'), 128.9 (C-3'/5'), 129.0 (C-4'), 167.0 (C=O), 164.4 (C=N), 135.7 (C=CH), 126.8 (C=CH), 135.1 (C-1''), 126.2 (C-2''), 148.2 (C-3''), 124.8 (C-4''), 130.4 (C-5''), 137.8 (C-6''). EIMS, 70 eV, m/z (rel. int. %): 460 (30, M^+), 243 (100), 271 (30), 432 (5). HR-ESIMS: calcd for $\text{C}_{27}\text{H}_{17}\text{N}_4\text{O}_4$: 461.1250 [$\text{M}+\text{H}$] $^+$; found: 461.1296.

2-[1-(4-Acetoxyphenyl)-9H- β -carbolin-3-yl]-4-(3-nitrobenzylidene)-4H-oxazol-5-one (9). Yield: 30%, m.p. 255–257 °C. IR (KBr) ν_{\max} cm^{-1} : 1816 cm^{-1} (C=O), 1655 (C=N), 1553–1528 (C=C). $^1\text{H-NMR}$ (DMSO- d_6): δ 9.20 (s, H-4), 8.51 (d, $J = 8.1$ Hz, H-5), 7.41 (t, $J = 8.1$ Hz, H-6), 7.67 (d, $J = 8.1$ Hz, H-7), 7.75 (d, $J = 8.1$ Hz, H-8), 12.26 (s, NH-9), 8.20 (d, $J = 8.4$ Hz, H-2'/6'), 7.45 (d, $J = 8.4$ Hz, H-3'/5'), 2.37 (s, CH_3), 7.56 (s, C=CH), 9.32 (s, H-2''), 8.34 (dd, $J = 7.8$ Hz and 2.1 Hz, H-4''), 7.87 (t, $J = 7.8$ Hz, H-5''), 8.83 (d, $J = 7.8$ Hz, H-6''). $^{13}\text{C-NMR}$ (DMSO- d_6): δ 142.3 (C-1), 141.5 (C-3), 117.0 (C-4), 129.1 (C-4a), 120.9 (C-4b), 122.1 (C-5), 121.0 (C-6), 129.4 (C-7), 113.0 (C-8), 144.1 (C-8a), 134.7 (C-9a), 132.3 (C-1'), 129.8 (C-2'/6'), 122.3 (C-3'/5'), 151.3 (C-4'), 20.9 (CH_3), 169.2 (OCOCH_3), 166.9 (C=O), 164.4 (C=N), 135.7 (C=CH), 126.4 (C=CH), 135.1 (C-1''), 126.2 (C-2''), 148.2 (C-3''), 124.8 (C-4''), 130.4 (C-5''), 137.8 (C-6''). HR-ESIMS: calcd for $\text{C}_{29}\text{H}_{19}\text{N}_4\text{O}_6$ [$\text{M}+\text{H}$] $^+$: 519.1305; found: 519.1400.

2-[1-(4-Methoxyphenyl)-9H- β -carbolin-3-yl]-4-(3-nitrobenzylidene)-4H-oxazol-5-one (10). Yield: 32%, m.p. 201–203 °C. IR (KBr) ν_{\max} cm^{-1} : 3330 (N–H), 1781 cm^{-1} (C=O), 1609 (C=N), 1547–1494 (C=C). $^1\text{H-NMR}$ (DMSO- d_6): δ 9.15 (s, H-4), 8.48 (d, $J = 7.6$ Hz, H-5), 7.40 (t, $J = 7.6$ Hz, H-6), 7.65 (t, $J = 7.6$ Hz, H-7), 7.71 (d, $J = 7.6$ Hz, H-8), 12.14 (s, NH-9), 8.12 (d, $J = 8.7$ Hz, H-2'/6'), 7.24 (d, $J = 8.7$ Hz, H-3'/5'), 3.91 (s, OCH_3), 7.55 (s, C=CH), 9.32 (s, H-2''), 8.35 (d, $J = 8.0$ Hz, H-4''), 7.87 (t, $J = 8.0$ Hz, H-5''), 8.51 (d, $J = 8.0$ Hz, H-6''). $^{13}\text{C-NMR}$ (DMSO- d_6): δ 143.1 (C-1), 138.0 (C-3), 116.6 (C-4), 129.2 (C-4a), 120.0 (C-4b), 122.0 (C-5), 121.0 (C-6), 128.9 (C-7), 109.9 (C-8), 142.0 (C-8a), 134.7 (C-9a), 129.6 (C-1'), 130.1 (C-2'/6'), 114.3 (C-3'/5'), 160.2 (C-4'), 55.4 (OCH_3), 167.1 (C=O), 164.5 (C=N), 135.8 (C=CH), 126.7 (C=CH), 135.2 (C-1''), 126.2 (C-2''), 148.2 (C-3''), 124.8 (C-4''), 131.0 (C-5''), 137.8 (C-6''). EIMS, 70 eV, m/z (rel. int. %): 490 (60, M^+), 301 (30), 273 (100), 258 (35). HR-ESIMS: calcd for $\text{C}_{28}\text{H}_{19}\text{N}_4\text{O}_5$ [$\text{M}+\text{H}$] $^+$: 491.1355; found: 491.1309.

2-[1-(4-Methoxyphenyl)-9H- β -carbolin-3-yl]-4-benzylidene-4H-oxazol-5-one (**11**). Yield: 40%, m.p. 245–247 °C. IR (KBr) ν_{\max} cm^{-1} : 3330 (N–H), 1781 cm^{-1} (C=O), 1648 (C=N), 1573–1494 (C=C). $^1\text{H-NMR}$ (DMSO- d_6): δ 9.18 (s, H-4), 8.55 (d, $J = 7.5$ Hz, H-5), 7.41 (m, H-6), 7.64 (t, $J = 7.5$ Hz, H-7), 7.74 (d, $J = 7.5$ Hz, H-8), 12.00 (s, NH-9), 8.06 (d, $J = 8.4$ Hz, H-2'/6'), 7.24 (d, $J = 8.4$ Hz, H-3'/5'), 3.90 (s, OCH₃), 7.56 (s, C=CH), 8.40 (d, $J = 7.2$ Hz, H-2''/6''), 7.58 (d, $J = 7.2$ Hz, H-3''/H-5''), 7.37 (m, H-4''). $^{13}\text{C-NMR}$ (DMSO- d_6): δ 143.4 (C-1), 138.2 (C-3), 116.7 (C-4), 129.6 (C-4a) 121.5 (C-4b), 122.7 (C-5), 121.1 (C-6), 129.4 (C-7), 113.4 (C-8), 141.9 (C-8a), 135 (C-9a), 133.1 (C-1'), 130.5 (C-2'/6'), 114.7 (C-3'/5'), 160.6 (C₀-4'), 55.5 (OCH₃), 168.0 (C=O), 164.0 (C=N), 134.1 (C=CH), 131.5 (C=CH), 134 (C-1''), 132.7 (C-2''/6''), 129.4 (C-3''/5''), 130.0 (C-4''). EIMS, 70 eV, m/z (rel. int. %): 445 (35, M⁺), 301 (30), 273 (100), 258 (35), 242 (15). HR-ESIMS: calcd for C₂₈H₂₀N₃O₃ [M+H]⁺: 446.1505; found: 446.1529.

3.2. Biological Assays

3.2.1. Cytotoxic Assay

The synthesized compounds were evaluated *in vitro* against six human cancer cell lines consisting of glioma (U251), melanoma (UACC-62), breast (MCF-7), prostate (PC-3), ovarian (OVCAR-03) and colon (HT-29). Cell lines were obtained from National Cancer Institute (Frederick, MD, USA). Normal cell line (VERO, renal, green monkey), from the Rio de Janeiro Cell Bank, was also used. Stock cultures were grown in medium containing 5 mL RPMI 1640 (GIBCO BRL) supplemented with 5% fetal bovine serum (FBS, GIBCO), at 37 °C with 5% CO₂. Penicillin:streptomycin (1000 $\mu\text{g/L}$:1000 U/L, 1 mL/L) was added to the experimental cultures. Cells in 96-well plates (100 μL cells well⁻¹) were exposed to compounds **8–11** in DMSO (concentrations of 0.25, 2.5, 25, and 250 $\mu\text{g}\cdot\text{mL}^{-1}$) at 37 °C, 5% of CO₂ in air for 48 h. Final DMSO concentration did not affect the cell viability. Afterwards cells were fixed with 50% trichloroacetic acid and cell proliferation determined by spectrophotometric quantification (540 nm) of cellular protein content by using sulforhodamine B assay and doxorubicin as the positive control [36]. Three measurements were obtained: at time zero (T₀, at the beginning of incubation) and 48 h post-incubation for compound-free (C) and tested (T) cells. Cell proliferation was determined according to the equation $100 \times [(T - T_0)/C - T_0]$, for $T_0 < T \leq C$, and $100 \times [(T - T_0)/T_0]$, for $T \leq T_0$ and a concentration-response curve for each cell line was plotted using software ORIGIN 8.0[®] (OriginLab Corporation). Using the concentration-response curve for each cell line, the IC₅₀ values (concentration that produces a 50% reduction in cellular growth when compared to untreated control cells) and IC₁₀₀ values (concentration that promotes total growth inhibition) were determined through non-linear regression analysis using software ORIGIN 8.0[®] (OriginLab Corporation) [37]. Compounds with IC₅₀ values < 100 μM were considered actives.

3.2.2. Antimicrobial Activity Assay

Antibacterial and antifungal assays for compounds **8–11** were carried out according to previously reported experimental protocols [38,39]. The bacterial strains were grown overnight at 36 °C in Nutrient Agar (Merck, Darmstadt, Germany), and the strains were grown in Sabouraud Dextrose Agar. Inoculum for the assays was prepared by diluting scraped cell mass in 0.85% NaCl solution, adjusted

to McFarland scale 0.5 and confirmed by spectrophotometrical reading at 580 nm. Cell suspensions were finally diluted to 10^4 UFC·mL⁻¹ for use in the activity assays. Minimal Inhibitory Concentration (MIC) tests were carried out using Müller-Hinton broth (bacteria) or RPMI-1640 (yeasts) on a tissue culture test plate (96 wells). Each compound was tested in duplicate. The stock solutions of compounds firstly in DMSO and subsequently in Tween 80 water solution (0.1%) were diluted and transferred into the first well, and serial dilutions were performed so that concentrations in the range of 250–1.6 µg·mL⁻¹ were obtained. Chloramphenicol and nystatin (Merck) were used as the reference antibiotic control. The inoculum was added to all wells and the plates were incubated at 36 °C for 24 h. Antibacterial activity was detected by adding 0.5% aqueous solution of ntriphenyltetrazolium chloride (TTC, 20 µL, Merck). MIC was defined as the lowest concentration of the compounds that inhibited visible growth, as indicated by TTC staining (dead cells are not stained by TTC). For antifungal activity evaluation, after the incubation period changes in the RPMI-1640 medium color were verified from pink (original color) to yellow. The change indicates an acidification from medium by the microorganisms' growth.

4. Conclusions

In conclusion, four 1-substituted β-carboline-3-(4-benzylidene)-4*H*-oxazol-5-ones **8–11** were prepared and assayed for their antitumor and antimicrobial activities. The compound 2-[1-(4-methoxyphenyl)-9*H*-β-carbolin-3-yl]-4-(benzylidene)-4*H*-oxazol-5-one (**11**) was the most active derivative, exhibiting potent cytotoxic activity against glioma (U251), prostate (PC-3) and ovarian (OVCAR-03) cancer cell lines with IC₅₀ values of 0.48, 1.50 and 1.07 µM, respectively. *In silico* studies indicate that compounds of this class are potential new anticancer drug candidates.

Supplementary Materials

Supplementary materials can be accessed at: <http://www.mdpi.com/1420-3049/17/3/6099/s1>.

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Conflict of Interest

The authors declare no conflict of interest.

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Sample Availability: Samples of the compounds **8–11** are available from the authors.

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