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Article

Synthesis, Antileishmanial Activity and Spin Labeling EPR Studies of Novel β-Carboline-Oxazoline and β-Carboline-Dihydrooxazine Derivatives

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A series of novel 1-(substituted-phenyl)-3-(4,5-dihydro-1,3-oxazol-2-yl)-9*H*-β-carboline (**8a**-**8i**) and 1-(substituted-phenyl)-3-(5,6-dihydro-4*H*-1,3-oxazin-2-yl)-9*H*-β-carboline (**9a**-**9h**) derivatives, as well as their respective *N*-(chloroalkyl)-1-(substituted-phenyl)-9*H*-β-carboline-3-carboxamide precursors (**6a**-**6i** and **7a**-**7h**), were synthesized and evaluated for their *in vitro* antileishmanial activity against promastigote and intracellular amastigote forms of *Leishmania amazonensis*. Compounds **8d**, **8i**, **9e** and **9h** exhibited significant activity for both promastigote and amastigote forms, with IC_{50} (50% inhibitory concentration) values ranging from 2.9 to 23.0 μM. In addition, spin label electron paramagnetic resonance (EPR) spectroscopy studies were carried out for the most active compounds against *L. amazonensis* promastigotes. The studies indicated that the tested compounds cause strong stiffness in the parasite plasma membrane and are capable of inducing internal metalloproteins oxidation of the parasite, resulting in their cross-linking to skeletal proteins. Compounds **8d** and **8i** produced the largest effect, showing that the presence of oxazoline group at C-3 of β-carboline nucleus is important for antileishmanial activity.

Keywords: β-carboline, 4,5-dihydro-1,3-oxazole, 5,6-dihydro-4*H*-1,3-oxazine, *Leishmania amazonensis*, electron paramagnetic resonance

Introduction

Leishmaniases are a group of diseases caused by protozoan parasites from more than 20 *Leishmania* species that cause a variety of clinical manifestations in humans, among them, there are three main forms: visceral (VL), cutaneous (CL) and mucocutaneous.[1] The *Leishmania amazonensis* species, for example, is responsible for the anergic diffuse cutaneous form and the cutaneous forms with disseminated lesions.[2] According to World Health Organization (WHO),[1] 97 countries and territories are endemic for leishmaniasis and it is estimated that between 600,000 to 1 million cases of CL occur worldwide annually. The current treatment for the leishmaniasis is mainly performed with pentavalent antimonials, amphotericin B, miltefosine and paromomycin. However, there is an increased incidence of treatment failure due to the toxicity and resistance exhibited by these drugs.[3] Besides this, no vaccines against *Leishmania* infections are available.[4] Therefore, it is of great importance to develop more active and less toxic compounds than the drugs used currently.

In the recent years, several studies have reported the antileishmanial activity of heterocyclic compounds,[3] including β-carbolines alkaloids.[5-20] Ashok *et al.*,[13] for example, described the antileishmanial activity against *Leishmania infantum* and *Leishmania donovani* of a series of (1-phenyl-9*H*-pyrido [3,4-*b*]indol-3-yl) (4-phenylpiperazin-1-yl)methanone derivatives. Among the derivatives assayed, compound **I** (Figure 1) displayed potent inhibition for both *Leishmania* species, with 50% effective concentration (EC_{50}) values ranging from 1.9 to 6.9 μM.

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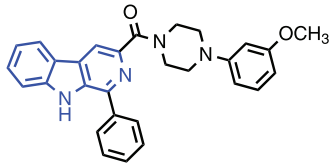
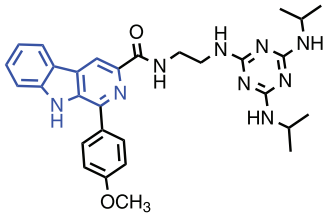
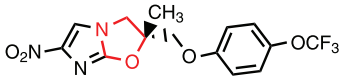


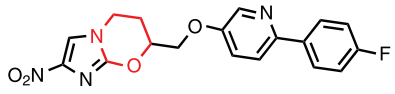
I
potent inhibition for *L. infantum* and *L. donovani*



II
potent activity against the promastigote and amastigote forms of *L. amazonensis*



III
candidate for the oral treatment of visceral leishmaniasis



IV
potent activity for *L. donovani* mouse model and *L. infantum* hamster model

Figure 1. Structures of β-carbolines (**I** and **II**), oxazoline (**III**) and dihydrooxazine (**IV**) derivatives with antileishmanial activity.

In this context, our research group has already demonstrated the antileishmanial activity of β-carbolines containing substituents at 1- and 3-positions of the β-carboline nucleus.[14-20] In the works developed by Tonin *et al.*[16] and Pedroso *et al.*,[17] it was demonstrated the activity of *N*-alkyl-(1-phenylsubstituted-β-carboline)-3-carboxamides against promastigote, axenic amastigote and intracellular amastigote forms of *Leishmania amazonensis*. The compound with the *N*-benzyl-carboxamide group at C-3 was active against promastigote and axenic amastigote forms with IC_{50} (50% inhibitory concentration) values of 2.6 and 1.0 μM, respectively,[17] and killed *L. amazonensis* promastigotes through different cell death pathways, including apoptosis and autophagy.[18] Recently, the antileishmanial activity of β-carboline-1,3,5-triazine hybrids was reported by Baréa *et al.*[19] Among the compounds tested, the hybrid **II** (Figure 1) showed potent activity against the promastigote (IC_{50} = 6.2 ± 1.4 μM, selectivity index (SI) = 23.5) and amastigote (IC_{50} = 1.2 ± 0.5 μM, SI = 121.4) forms of *L. amazonensis* and exhibited low toxicity. Studies of action mechanism in promastigotes showed that compound **II** caused alterations in cell division cycle and an increase of lipid-storage bodies, leading the cells to death through various factors. The accumulation of lipid bodies may be associated with apoptotic cell death.[19]

Additionally, oxazoline and 5,6-dihydro-4*H*-1,3-oxazine heterocycles play an important role in organic synthesis, being present in the structure of various biologically active compounds.[20-27] For instance, the nitroimidazo-oxazole **III** (Figure 1) showed IC_{50} of 0.03 μM against the amastigote form of *L. donovani* DD8 transfected with luciferase, and was identified, from a series of 72 nitroimidazoles evaluated, as a candidate for the oral treatment of visceral leishmaniasis. This compound showed also *in vivo* activity in both rat and hamster models.[25] The dihydrooxazine phenylpyridine **IV** (Figure 1) was effective for *L. donovani* mouse model and *L. infantum* hamster model, displaying optimal efficacy, pharmacokinetic and safety, leading to its selection as a new candidate for treatment of VL.[26]

Considering the promising antileishmanial properties of β-carboline nucleus, the synthetic and biological importance of oxazoline and 5,6-dihydro-4*H*-1,3-oxazine rings, and the need to develop antileishmanial agents more effective, in this work we designed new 1-(substituted-phenyl)-β-carboline derivatives bearing oxazoline and 5,6-dihydro-4*H*-1,3-oxazine moieties at C-3 (Figure 2). The novel 1,3-disubstituted-β-carboline derivatives were evaluated against promastigote and intracellular amastigote forms of *L. amazonensis* and their cytotoxicity were determined. The antileishmanial activity of *N*-(chloroalkyl)-β-carboline intermediates, precursors of the proposed derivatives, was also evaluated in order to verify the importance of the heterocyclic ring at the 3-position of β-carboline nucleus.

In addition, electron paramagnetic resonance (EPR) spectroscopy, associated with spin labeling method studies, was carried out for the most active compounds against *L. amazonensis* promastigotes. This analysis has been shown to be an important tool for analyzing the interaction of drugs or prototypes of drugs with parasite membranes. The literature describes the employment of this technique to evaluate the effects of miltefosine,[28,29] nerolidol[30,31] and parthenolide[32] on *L. amazonensis* membrane, and of elatol on *Trypanosoma cruzi*.[33]

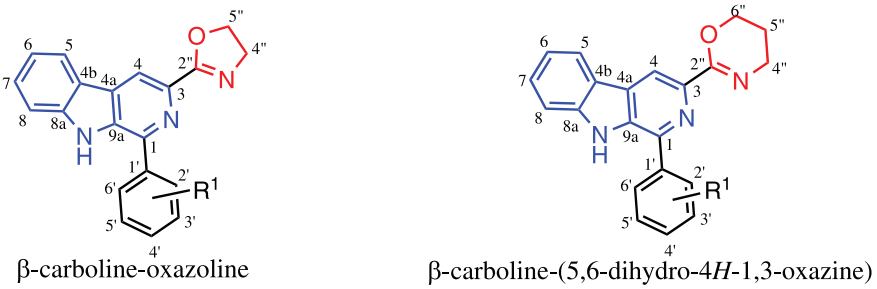


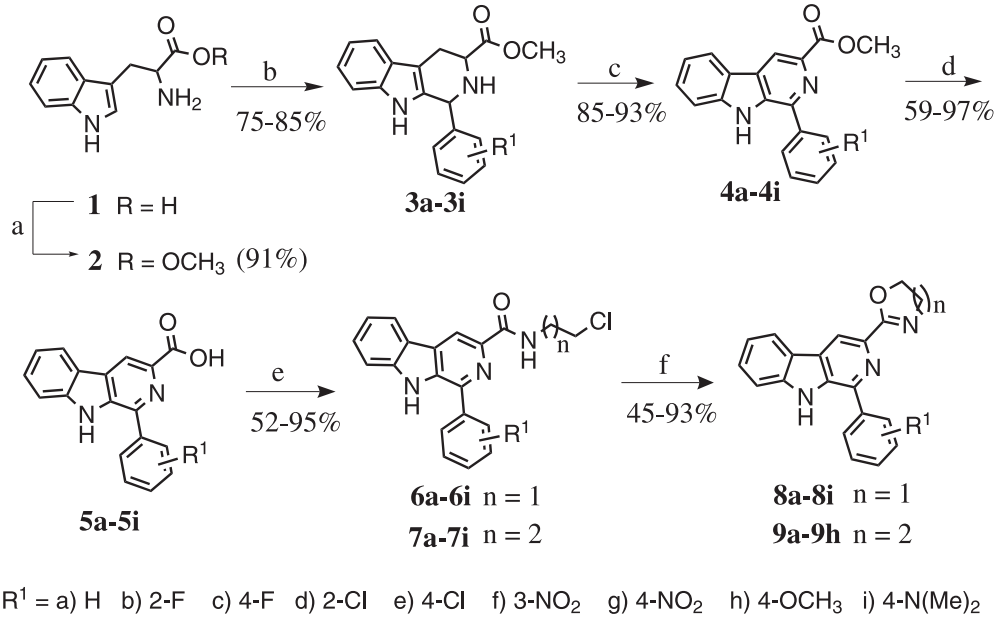
Figure 2. Structures of novel β-carboline-oxazoline and β-carboline-(5,6-dihydro-4*H*-1,3-oxazine) derivatives proposed.

Results and Discussion

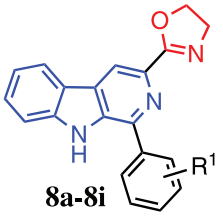
Chemistry

Novel 1-(substituted-phenyl)-β-carboline derivatives bearing oxazoline (**8a**-**8i**) and 5,6-dihydro-4*H*-1,3-oxazine (**9a**-**9h**) moieties at C-3 were synthesized from the *N*-(chloroalkyl)-β-carboline-3-carboxamides **6a**-**6i** and **7a**-**7h** as shown in Scheme 1. The β-carboline nucleus was prepared from commercial L-tryptophan (**1**) according to the methodology described by our research group.[14-19] The L-tryptophan methyl ester (**2**) was subjected to the condensation reaction of Pictet-Spengler with different aldehydes in acid medium, providing the methyl 1-(substituted-phenyl)-1,2,3,4-tetrahydro-9*H*-β-carboline-3-carboxylates **3a**-**3i**, which were oxidized with sulfur under reflux in xylene to the methyl 1-(substituted-phenyl)-9*H*-β-carboline-3-carboxylates **4a**-**4i**. The basic hydrolysis reaction of **4a**-**4i** provided the 1-(substituted-phenyl)-9*H*-β-carboline-3-carboxylic acids **5a**-**5i**. The intermediates **5a**-**5i** were subjected to the nucleophilic substitution reaction with 2-chloroethylamine or 3-chloropropylamine, using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) as carboxylic acid activator, providing the *N*-(chloroalkyl)-β-carbolines **6a**-**6i** and **7a**-**7i** with yields in the range of 52 to 95%.

The β-carboline-oxazoline **8a**-**8i** derivatives were obtained in 46-93% yield (Figure 3) from the nucleophilic cyclo-*O*-alkylation of **6a**-**6i** in refluxing dimethylformamide (DMF), using potassium carbonate as base. The intramolecular *O*-cyclization of **7a**-**7h** using DMF under microwave irradiation and K_2CO_3, afforded the β-carboline-dihydrooxazine **9a**-**9h** derivatives with yields in the range of 45 to 86% (Figure 3). The intermediate **7i** was also submitted to cyclization reaction, under the similar conditions employed for **7a**-**7h**; however, in this case, the product formed underwent decomposition during workup.

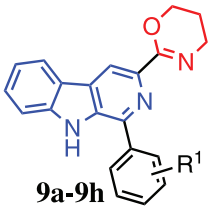


Scheme 1. Synthesis of derivatives **8a**-**8i** and **9a**-**9h**. Reagents and conditions: (a) MeOH, H_2SO_4 (cat), reflux, 48 h; (b) R^1Ph-CHO, trifluoroacetic acid (TFA), CH_2Cl_2, room temperature (rt), 18-26 h; (c) sulfur, xylene, reflux, 48 h; (d) NaOH, MeOH/H_2O (2:1), reflux, 6-18 h; (e) 2-chloroethylamine hydrochloride (for **6a**-**6i**) or 3-chloropropylamine hydrochloride (for **7a**-**7i**), Et_3N, DMTMM, THF:MeOH (8:2); (f) (*i*) K_2CO_3, DMF, reflux, 12-20 h for **8a**-**8i**; (*ii*) K_2CO_3, DMF, MW (100% power level), 5-9 min for **9a**-**9h**.



8a-8i

Compound	yield / %	reaction time / h	Compound	yield / %	reaction time / h
8a	85	18	**8f**	58	15
8b	46	18	**8g**	74	15
8c	51	12	**8h**	81	11
8d	65	16	**8i**	93	12
8e	50	20			



9a-9h

Compound	yield / %	reaction time / min	Compound	yield / %	reaction time / min
9a	45	7	**9e**	60	7
9b	73	9	**9f**	51	7
9c	45	8	**9g**	51	5
9d	86	7	**9h**	66	6

R^1 = a) H b) 2-F c) 4-F d) 2-Cl e) 4-Cl f) 3-NO_2 g) 4-NO_2 h) 4-OCH_3 i) 4-$N(Me)_2$

Figure 3. Yields and reaction times for preparation of **8a**-**8i** and **9a**-**9h** from cyclo-*O*-alkylation of **6a**-**6i** and **7a**-**7h**.

The β-carboline-oxazoline **8a**-**8i** and β-carboline-dihydrooxazine **9a**-**9h** derivatives were characterized by their spectral data (high-resolution mass spectrometry (HRMS), ^{1}H and ^{13}C nuclear magnetic resonance (NMR)). The formation of the **8a**-**8i** was supported by the absence of the signal at δ_H 8.54-9.01 referent to the NH of the carboxamide group and the presence of two triplets at δ_H 4.06-4.19 (CH_2, C-4") and 4.50-4.59 (CH_2, C-5"), in ^{1}H NMR spectra. The presence of oxazoline ring was also confirmed by the signals at δ_C 54.4-55.1 (CH_2, C-4") and 67.4-68.4 (CH_2, C-5"), in ^{13}C NMR spectra. The 5,6-dihydro-4*H*-1,3-oxazine heterocycle at 3-positon of β-carboline nucleus in compounds **9a**-**9h** were confirmed by the signals at δ_H 1.93-2.07 (quintet, CH_2), 3.55-3.74 (triplet, CH_2) and 3.83-4.51 (triplet, CH_2), in ^{1}H NMR spectra, and signals at δ_C 21.6-22.0 (CH_2), 42.1-43.0 (CH_2) and 64.7-65.7 (CH_2), in ^{13}C NMR spectra.

Antileishmanial activity

The β-carboline-oxazolines **8a**-**8i** and β-carboline-dihydrooxazines **9a**-**9h** were evaluated *in vitro* against the promastigote form of *L. amazonensis* (Table 1). The compounds that showed IC_{50} values greater than 100 μM were considered inactive. For the most active compounds against promastigotes, the antileishmanial activity for the intracellular amastigote form of *L. amazonensis* was also evaluated. The toxic effects on the host cells were determined by the selectivity index (SI). The SI for each active compound was calculated as the ratio between the cytotoxicity (CC_{50}) for macrophage J774-A1 cell lines and IC_{50} against the promastigote and intracellular amastigote forms of *L. amazonensis*.

Analysis of the IC_{50} values (Table 1) for β-carboline-oxazolines **8a**-**8i** shows that the presence of chlorine and dimethylamino substituents, at 2- and 4-positions of phenyl group linked to C-1, led to the active compounds **8d** and **8i**, respectively. Compound **8d** showed also better selectivity indices (SI) for both forms of *L. amazonensis* than for the host cells (Table 1), being the most promising compound in this series.

Concerning to **9a**-**9h** series, most of β-carboline-dihydrooxazine derivatives showed moderate activity for *L. amazonensis* promastigotes, with IC_{50} values ranging from 21.3 to 58.0 μM (Table 1). The derivatives **9a**, **9e** and **9h** containing the phenyl, 4-chlorophenyl and 4-methoxyphenyl substituents, respectively, at C-1 of β-carboline nucleus, were the most active compounds for promastigote form, exhibiting IC_{50} values in the range of 21.3 to 27.5 μM, similar to that of reference drug miltefosine.[34] These compounds were then evaluated against intracellular amastigote form of *L. amazonensis* and showed IC_{50} values in the range of 2.9 to 75.5 μM (Table 1). The derivative **9e** was the most promising, being 26 and 6 times more active than **9a** and **9h**, respectively. Besides that, **9e** was 29.7 times more toxic for intracellular amastigotes than for macrophage J774-A1 cell lines, being a promising antileishmanial agent.

Comparison of the IC_{50} data of the novel 1,3-disubstituted-β-carboline derivatives, **8a**-**8h** and **9a**-**9h** showed that except for compound **8d**, the replacement of oxazoline by 5,6-dihydro-4*H*-1,3-oxazine ring led to an enhancement of the antileishmanial activity.

In this work, we also compared the antileishmanial activity of the β-carboline derivatives **8a**-**8i** and **9a**-**9h** with their intermediates *N*-(chloroalkyl)-β-carboline **6a**-**6i** and **7a**-**7h**, respectively (Table 2). Among the *N*-(chloroalkyl)-

Table 1. Antileishmanial activity data for derivatives **8a**-**8i** and **9a**-**9h** against *L. amazonensis*

Compound	IC_{50pro} / μM	IC_{50ama} / μM	CC_{50} / μM	SI_{pro}	SI_{ama}
8a	> 100	n.t	270.0 ± 28.3	n.d	n.d
8b	> 100	n.t	112.5 ± 24.7	n.d	n.d
8c	> 100	n.t	95.0 ± 5.0	n.d	n.d
8d	14.7 ± 6.7	10.5 ± 3.2	96.5 ± 10.6	6.6	9.2
8e	> 100	n.t	43.0 ± 18.4	n.d	n.d
8f	> 100	n.t	170.5 ± 28.9	n.d	n.d
8g	> 100	n.t	88.0 ± 10.6	n.d	n.d
8h	88.5 ± 10.6	n.t	249.5 ± 13.5	2.9	n.d
8i	23.0 ± 7.1	12.3 ± 3.1	30.5 ± 5.3	1.3	2.5
9a	27.5 ± 6.4	75.5 ± 6.7	67.5 ± 17.7	2.5	0.9
9b	53.5 ± 17.7	n.t	73.2 ± 16.3	1.4	n.d
9c	57.3 ± 11.2	n.t	120.0 ± 35.0	2.1	n.d
9d	58.0 ± 2.8	n.t	62.8 ± 24.7	1.1	n.d
9e	22.7 ± 2.1	2.9 ± 0.8	86.0 ± 12.1	3.8	29.7
9f	> 100	n.t	95.3 ± 30.7	n.d	n.d
9g	> 100	n.t	58.0 ±13.8	n.d	n.d
9h	21.3 ± 4.2	17.0 ± 1.4	39.5 ± 3.55	1.9	2.3
Miltefosine[a]	18.5 ± 1.1	2.4 ± 0.1	40.5 ± 1.7	2.2	16.9

[a]Positive control.[34] IC_{50pro}: 50% inhibitory concentration against promastigotes; IC_{50ama}: 50% inhibitory concentration against intracellular amastigotes; CC_{50}: 50% cytotoxic concentration; SI_{pro}: selectivity index for promastigotes; SI_{ama}: selectivity index for intracellular amastigotes; n.t: not tested; n.d: not determined.

β-carbolines evaluated, only **6d** was active, showing significant activity against both forms of *L. amazonensis* (IC_{50pro} = 2.2 ± 1.3 μM; IC_{50ama} = 6.3 ± 0.8 μM). These results demonstrate that the presence of oxazoline and dihydrooxazine rings in the 3-position of the β-carboline nucleus is important for antileishmanial activity.

Spin label EPR spectroscopy studies

In order to investigate the interaction of the most active compounds for the promastigote form of *L. amazonensis* with the parasite membrane, EPR spectroscopy associated with the spin labeling method studies were carried out for **6d**, **8d**, **8i**, **9a**, **9e** and **9h**. Figure 4 shows the EPR spectra of the spin label 5-doxyl-stearic acid (5-DSA) incorporated in *Leishmania* membranes for samples untreated and treated with the studied compounds. EPR spectra showed that all compounds cause increases in parameter $2A_{//}$ (outer hyperfine splitting) above the estimated experimental error (0.5 G), indicating decreases in molecular dynamics. In the treatment with 150 μM of compounds, some of them showed remarkable changes in the parasite membrane. Compounds **8d** and **8i** containing the oxazoline heterocycle at 3-position of β-carboline nucleus were the most effective for treatments at a concentration of 150 μM. However, we note that the compounds **6d**, **9a**, **9e** and **9h** can also cause high membrane stiffness at higher concentrations. In cell membrane the probe 5-DSA behaves as annular or boundary lipids that preferentially surround the hydrophobic surface of membrane proteins.[35] Because of these interactions with the transmembrane proteins, 5-DSA can monitor the dynamics at the periphery of proteins into the lipid bilayer. Thus, the changes in 5-DSA spectra caused by the compounds may be associated with changes in the membrane protein component.

Spin label EPR spectroscopy indicated that the treatments of *L. amazonensis* promastigotes with the studied compounds cause strong stiffness in the parasite plasma membrane. These strong membrane changes, with changes in parameter $2A_{//}$ of ca. 5 G observed for two compounds at a relatively low concentration, cannot be explained by the simple presence of the molecules in the membrane, but must involve some oxidation process. Similar alterations in the EPR spectra of 5-DSA into plasma membrane were found in a previous study[36] for erythrocytes oxidized with hydrogen peroxide in phosphate buffer with azide (catalase inhibitor). EPR spectra of erythrocyte membrane spin-labeled with 5-DSA showed a change in parameter $2A_{//}$ of 57.5 G for untreated erythrocyte (control) to 60.5 G for erythrocyte treated with 200 μM H_2O_2. It

Table 2. Antileishmanial activity data for compounds **6a**-**6i** and **7a**-**7i** against *L. amazonensis*

Compound	IC_{50pro} / μM	IC_{50ama} / μM	CC_{50} / μM	SI_{pro}	SI_{ama}
6a	> 100	n.t	175.0 ± 35.4	n.d	n.d
6b	> 100	n.t	49.6 ± 16.3	n.d	n.d
6c	> 100	n.t	161.7 ± 7.6	n.d	n.d
6d	2.2 ± 1.3	6.3 ± 0.8	19.0 ± 6.2	8.7	3.0
6e	> 100	n.t	127.0 ± 32.5	n.d	n.d
6f	> 100	n.t	201.0 ± 9.9	n.d	n.d
6g	> 100	n.t	97.0 ± 4.2	n.d	n.d
6h	> 100	n.t	68.5 ± 10.6	n.d	n.d
6i	> 100	n.t	30.0 ± 3.0	n.d	n.d
7a	> 100	n.t	630.0 ± 169.0	n.d	n.d
7b	92.0 ± 4.05	n.t	83.3 ± 20.5	n.d	n.d
7c	> 100	n.t	70.7 ± 16.2	n.d	n.d
7d	> 100	n.t	< 10	n.d	n.d
7e	> 100	n.t	365.0 ± 120.2	n.d	n.d
7f	> 100	n.t	273.3 ± 59.6	n.d	n.d
7g	> 100	n.t	895.0 ± 148.0	n.d	n.d
7h	> 100	n.t	290.0 ± 88.2	n.d	n.d
7i	> 100	n.t	615.0 ± 49.5	n.d	n.d
Miltefosine[a]	18.5 ± 1.1	2.4 ± 0.1	40.5 ± 1.7	2.2	16.9

[a]Positive control;[34] IC_{50pro}: 50% inhibitory concentration against promastigotes; IC_{50ama}: 50% inhibitory concentration against intracellular amastigotes; CC_{50}: 50% cytotoxic concentration; SI_{pro}: selectivity index for promastigotes; SI_{ama}: selectivity index for intracellular amastigotes; n.t: not tested; n.d: not determined.

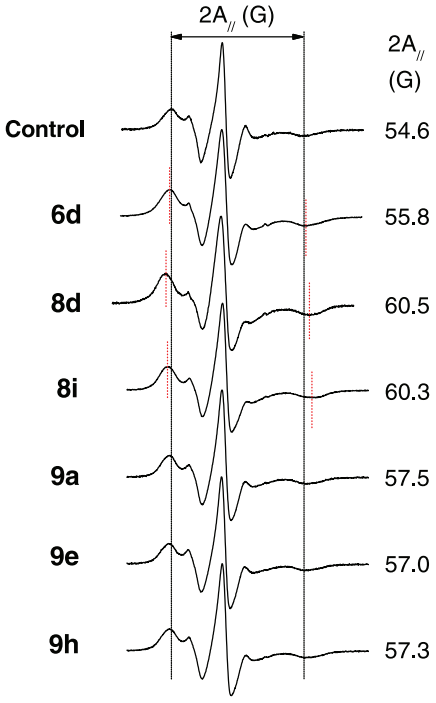


Figure 4. EPR spectra of spin label 5-DSA incorporated in *Leishmania amazonensis* promastigotes membranes for samples of untreated cells (control) and treated with several compounds (150 μM). The values of the EPR parameter $2A_{//}$ (outer hyperfine splitting), which is given by the separation in magnetic field units between the first peak and the last inverted peak of the spectrum, are indicated. The estimated experimental error for the $2A_{//}$ parameter is 0.5 G. The total scan range of the magnetic field in each EPR spectrum was of 100 G (*x* axis) and the intensity (*y* axis) is in arbitrary units.

has been shown that H_2O_2 induces the formation of cross-linking of hemoglobin to skeletal proteins in the membranes of human erythrocytes in an azide phosphate buffer, associated with a progressive alteration of the cell's shape to echinocytic morphology, decreased cell deformability and increased phagocytosis.[37] Heme proteins were crucial for the occurrence of these cellular alterations, since they may be completely inhibited by previous exposure of red blood cells to carbon monoxide. Lipid peroxidation did not appear to be important because the antioxidant butylated hydroxytoluene decreased the fluorescent derivatives but did not prevent formation of the spectrin-Hb (hemoglobin) complex.[37] These observations suggest that the compounds tested are capable of inducing oxidation of internal metalloproteins of the parasite, resulting in their cross-linking to skeletal proteins.

Conclusions

Novel β-carboline-oxazoline **8a**-**8i** and β-carboline-dihydrooxazine **9a**-**9h** derivatives have been synthesized in moderate to good yields (45-93%) from the nucleophilic cyclo-*O*-alkylation of intermediates *N*-(chloroalkyl)-β-carboline-3-carboxamide **6a**-**6i** and **7a**-**7h**, respectively. Compounds **6d**, **8d**, **8i**, **9e** and **9h** showed significant activity in *L. amazonensis* promastigotes (IC_{50} values ranging from 2.2 to 23.0 μM), and also in intracellular

amastigote forms (IC_{50} values ranging from 2.9 to 17.0 μM). Compound **9e** was the most active for intracellular amastigotes (IC_{50} = 2.9 ± 0.8 μM) and showed also low toxicity, being 29.7 times more toxic for intracellular amastigotes than for macrophage J774-A1 cell lines.

The antileishmanial activity data showed that the presence of oxazoline and 5,6-dihydro-4*H*-1,3-oxazine moieties at C-3 of β-carboline nucleus led to an increase of compounds number with antileishmanial activity, in comparison to the *N*-(chloroalkyl)-β-carboline-3-carboxamide precursors. Comparison of the IC_{50} data of the compounds **8a**-**8h** and **9a**-**9h** showed that except for compound **8d**, the replacement of oxazoline by 5,6-dihydro-4*H*-1,3-oxazine ring led to an enhancement of the antileishmanial activity.

Spin label EPR spectroscopy studies indicated that the tested compounds cause strong stiffness in the parasite plasma membrane and are capable of inducing internal metalloproteins oxidation of the parasite, resulting in their cross-linking to skeletal proteins. Compounds **8d** and **8i** produced the largest effect, showing that the presence of oxazoline group at C-3 of β-carboline nucleus is important for antileishmanial activity. Further studies will be conducted with these compounds aiming a better understanding of their mechanisms of action. Compounds **8d** and **8i** are also strong candidates for *in vivo* studies in view to the development of new antileishmanial agents.

Experimental

General methods

All reagents were purchased from commercial suppliers, except the DMTMM that was synthesized according to the methodology described by Cronin *et al*.[38] and Kunishima *et al*.[39] The reactions were monitored by thin layer chromatography (TLC) conducted on Whatman TLC plates (silica gel 60 F_{254}). NMR spectra were recorded in a Varian spectrometer model Mercury plus BB at 300 (for ^{1}H) and 75 MHz (for ^{13}C) and in a Bruker spectrometer model Avance III HD at 500 (for ^{1}H) and 125 MHz (for ^{13}C), with deuterated solvents, chloroform ($CDCl_3$), methanol (CD_3OD) and dimethyl sulfoxide (DMSO-d_6), and tetramethylsilane (TMS) as internal standard. Mass spectra (electrospray ionization mass spectrometry (ESI-MS)) were recorded on Thermoelectron Corporation Focus-DSQ II spectrometer. Melting points were determined in Microquímica apparatus model MQAPF-301 and are uncorrected. Spin label 5-DSA was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Chemistry

Synthesis of **5a**-**5i**

The intermediates **5a**-**5i** were synthesized according to the protocol described previously by our research group.[16,17,19] To a suspension of 1-(substituted-phenyl)-β-carboline-3-carboxylates **4a**-**4i** (1 mmol) in methanol-water (2:1), it was added 4 mmol of sodium hydroxide (0.16 g). The mixture was refluxed until complete consumption of **4a**-**4i** (6-18 h). Then, the reaction mixture was cooled, treated with an HCl solution (2 M) until pH 5 and left on ice bath for 2 h. The precipitate formed was filtered and washed with distilled water. The β-carboline-carboxylic acids **5a**-**5i** were obtained in yields in the range 59-97%.

Synthesis of *N*-(2-chloroethyl)-1-(substituted-phenyl)-9*H*-β-carboline-3-carboxamides (**6a**-**6i**) and *N*-(3-chloropropyl)-1-(substituted-phenyl)-9*H*-β-carboline-3-carboxamides (**7a**-**7i**)

To a solution of β-carboline-carboxylic acids **5a**-**5i** (1 mmol) in tetrahydrofuran/methanol 8:2 (10 mL), it was added 1.5 mmol of 2-chloroethylamine hydrochloride (0.17 g) or 3-chloropropylamine hydrochloride (0.20 g), 1.5 mmol of triethylamine (0.21 mL) and 1.5 mmol of DMTMM (0.36 g). The reaction mixture was stirred at room temperature until complete consumption of **5a**-**5i** (14 to 20 h). After this reaction time, the solvent was removed in a rotary evaporator and the residue solubilized in 1 mL of ethanol. Then, distilled water was added to the solution and the precipitate formed was filtered under vacuum and washed with distilled water. The compounds **6a**-**6i** and **7a**-**7i** were obtained in yields in the range of 52-95%.

N-(2-Chloroethyl)-1-phenyl-9*H*-β-carboline-3-carboxamide (**6a**)

Yield: 67%; mp 177-180 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 3.76 (t, *J* 5.8 Hz, 2H, H-2"), 3.87 (q, *J* 5.8 Hz, 2H, H-1"), 7.35 (t, *J* 7.9 Hz, 1H, H-6), 7.50-7.64 (m, 5H, H-7, H-8, H-3', H-4', H-5'), 7.99 (d, *J* 7.2 Hz, 2H, H-2', H-6'), 8.18 (d, *J* 7.9 Hz, 1H, H-5), 8.66 (t, 1H, *J* 5.8 Hz, CONH), 8.87 (s, H-4), 8.93 (s, 1H, NH, H-9); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 41.3 (CH_2, C-1"), 43.9 (CH_2, C-2"), 111.8 (CH, C-8), 113.6 (CH, C-4), 121.1 (CH, C-6), 122.2 (CH, C-5), 122.2 (C_0, C-4b), 128.2 (2CH, C-2', C-6'), 129.0 (CH, C-4'), 129.3 (CH, C-7), 129.3 (CH, C-3', C-5'), 130.6 (C_0, C-4a), 134.9 (C_0, C-9a), 137.8 (C_0, C-1'), 140.0 (C_0, C-3), 140.7 (C_0, C-1), 141.1 (C_0, C-8a), 165.8 (C=O); HRMS-ESI *m/z*, calcd. for $C_{20}H_{17}ClN_3O$ $[M + H]^+$: 350.1055, found: 350.1049.

N-(2-Chloroethyl)-1-(2-fluorophenyl)-9*H*-β-carboline-3-carboxamide (**6b**)

Yield: 75%; mp 171-172 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 3.77 (t, *J* 5.8 Hz, 2H, H-2"), 3.89 (q, *J* 5.8 Hz, 2H, H-1"), 7.29-7.44 (m, 3H, H-6, H-3', H-6'), 7.50-7.63 (m, 3H, H-4', H-7, H-8), 7.90 (td, *J* 7.6, 1.7 Hz, 1H, H-5'), 8.23 (d, *J* 7.9 Hz, 1H, H-5), 8.58-8.63 (m, 2H, CONH, NH, H-9), 8.95 (s, 1H, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 41.2 (CH_2, C-1"), 43.9 (CH_2, C-2"), 111.8 (CH, C-8), 114.1 (CH, C-4), 116.3 (CH, d, *J* 22.5 Hz, C-3'), 121.0 (CH, C-6), 122.0 (C_0, C-4b), 122.2 (CH, C-5), 125.2 (CH, C-6'), 125.3 (C_0, C-4a), 129.1 (CH, C-7), 130.6 (C_0, C-1'), 131.2 (C_0, d, *J* 8.4 Hz, C-4'), 132.3 (CH, d, *J* 3.7 Hz, C-5'), 135.6 (C_0, C-9a), 136.3 (C_0, C-3), 140.1 (C_0, C-1), 140.6 (C_0, C-8a), 159.9 (C_0, d, *J* 246 Hz, 1C, C-2'), 165.6 (C=O); HRMS-ESI *m/z*, calcd. for $C_{20}H_{16}ClFN_3O$ $[M + H]^+$: 368.0960, found: 368.0963.

N-(2-Chloroethyl)-1-(4-fluorophenyl)-9*H*-β-carboline-3-carboxamide (**6c**)

Yield: 91%; mp 164-167 °C; ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD) δ_H 3.77 (t, *J* 6.6 Hz, 2H, H-2"), 3.87 (m, 2H, H-1"), 7.27-7.36 (m, 3H, H-6, H-3', H-5'), 7.58 (m, 2H, H-7, H-8), 7.99 (dd, *J* 8.7, 5.3 Hz, 2H, H-2', H-6'), 8.17 (d, *J* 7.8 Hz, 1H, H-5), 8.67 (t, *J* 5.8 Hz, CONH), 8.82 (s, 1H, H-4), 9.81 (s, 1H, NH, H-9); ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD) δ_C 41.2 (CH_2, C-1"), 43.8 (CH_2, C-2"), 112.0 (CH, C-8), 113.6 (CH, C-4), 116.1 (d, *J* 21.9 Hz, 2CH, C-3', C-5'), 120.9 (CH, C-6), 122.0 (CH, C-5), 122.1 (C_0, C-4b), 128.9 (CH, C-7), 130.2 (d, *J* 8.5 Hz, 2 CH, C-2', C-6'), 130.7 (C_0, C-4a), 134.0 (C_0, C-9a), 135.0 (C_0, C-1'), 139.4 (C_0, C-3), 140.3 (C_0, C-1), 141.2 (C_0, C-8a), 163.3 (d, *J* 247.7 Hz, C_0, C-4'), 166.1 (C=O); HRMS-ESI *m/z*, calcd. for $C_{20}H_{16}ClFN_3O$ $[M + H]^+$: 368.0960, found: 368.0957.

N-(2-Chloroethyl)-1-(2-chlorophenyl)-9*H*-β-carboline-3-carboxamide (**6d**)

Yield: 94%; mp 246-248 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 3.76 (t, *J* 5.8 Hz, 2H, H-2"), 3.86 (q, *J* 5.8 Hz, 2H, H-1"), 7.37 (t, *J* 7.8 Hz, 1H, H-6), 7.47-7.68 (m, 6H, H-7, H-8, H-3', H-4', H-5', H-6'), 8.23 (d, *J* 7.8 Hz, 1H, H-5), 8.54 (m, 2H, CONH, NH, H-9), 8.95 (s, 1H, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 41.4 (CH_2, C-1"), 43.7 (CH_2, C-2"), 111.8 (CH, C-8), 114.2 (CH, C-4), 121.0 (CH, C-6), 122.1 (C_0, C-4b), 122.2 (CH, C-5), 127.4 (CH, C-7), 129.1 (CH, C-5'), 130.2 (C_0, C-4a), 130.4 (CH, C-3'), 130.5 (CH, C-6'), 131.9 (CH, C-4'), 133.0 (C_0, C-2'), 135.4 (C_0, C-9a), 136.3 (C_0, C-1'), 139.5 (C_0, C-3), 139.8 (C_0, C-1), 140.7 (C_0, C-8a), 165.7 (C=O); HRMS-ESI *m/z*, calcd. for $C_{20}H_{16}Cl_2N_3O$ $[M + H]^+$: 384.0665, found: 384.0665.

N-(2-Chloroethyl)-1-(4-chlorophenyl)-9*H*-β-carboline-3-carboxamide (**6e**)

Yield: 68%; mp 269-272 °C; ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD) δ_H 3.78 (t, *J* 5.8 Hz, 2H, H-2"), 3.88 (t, *J* 5.8 Hz, 2H, H-1"), 7.33-7.36 (m, 1H, H-6), 7.59 (m, 4H, H-7, H-8, H-3', H-5'), 7.98 (d, *J* 8.1 Hz, 2H, H-2', H-6'), 8.20 (d, *J* 7.9 Hz, 1H, H-5), 8.83 (s, 1H, H-4); ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD) δ_C 41.3 (CH_2, C-1"), 43.8 (CH_2, C-2"), 112.2 (CH, C-8), 113.8 (CH, C-4), 120.9 (CH, C-6), 122.0 (CH, C-5), 122.0 (C_0, C-4b), 129.0 (CH, C-7), 129.3 (2CH, C-3', C-5'), 129.8 (2CH, C-2', C-6'), 130.8 (C_0, C-4a), 135.0 (C_0, C-4'), 135.2 (C_0, C-9a), 136.4 (C_0, C-1'), 139.3 (C_0, C-3), 140.1 (C_0, C-1), 141.4 (C_0, C-8a), 166.3 (C=O); HRMS-ESI *m/z*, calcd. for $C_{20}H_{16}Cl_2N_3O$ $[M + H]^+$: 384.0665, found: 384.0673.

N-(2-Chloroethyl)-1-(3-nitrophenyl)-9*H*-β-carboline-3-carboxamide (**6f**)

Yield: 72%; mp 186-190 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 3.70-3.84 (m, 4H, H-1", H-2"), 7.35 (td, *J* 7.4, 1.1 Hz, 1H, H-6), 7.61-7.71 (m, 2H, H-7, H-8), 7.96 (t, *J* 8.0 Hz, 1H, H-5'), 8.40-8.48 (m, 2H, H-5, H-4'), 8.59 (dt, *J* 8.0, 1.1 Hz, 1H, H-6'), 8.86 (t, *J* 1.9 Hz, 1H, H-2'), 8.93 (s, 1H, H-4), 9.01 (t, *J* 5.8 Hz, 1H, CONH), 12.06 (s, 1H, NH, H-9); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 41.0 (CH_2, C-1"), 43.3 (CH_2, C-2"), 112.6 (CH, C-8), 114.1 (CH, C-4), 120.5 (CH, C-6), 121.2 (C_0, C-4b), 122.3 (CH, C-5), 123.5 (CH, C-2'), 123.6 (CH, C-4'), 129.0 (CH, C-7), 130.4 (C_0, C-4a), 130.4 (CH, C-5'), 134.5 (CH, C-6'), 135.4 (C_0, C-9a), 138.3 (C_0, C-1'), 138.9 (C_0, C-3), 139.7 (C_0, C-1), 141.7 (C_0, C-8a), 148.3 (C_0, C-3'), 164.9 (C=O); HRMS-ESI *m/z*, calcd. for $C_{20}H_{16}ClN_4O_3$ $[M + H]^+$: 395.0905, found: 395.0905.

N-(2-Chloroethyl)-1-(4-nitrophenyl)-9*H*-β-carboline-3-carboxamide (**6g**)

Yield: 79%; mp > 279 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 3.73-3.84 (m, 4H, H-1", H-2"), 7.35 (td, *J* 7.4, 1.1 Hz, 1H, H-6), 7.61-7.72 (m, 2H, H-7, H-8), 8.45-8.47 (m, 5H, H-5, H-2', H-3', H-5', H-6'), 8.94 (s, 1H, H-4), 9.01 (t, *J* 5.8 Hz, CONH), 12.07 (s, 1H, NH, H-9); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 41.0 (CH_2, C-1"), 43.2 (CH_2, C-2"), 112.6 (CH, C-8), 114.3 (CH, C-4), 120.5 (CH, C-6), 121.1 (CH, C-5), 122.3 (C_0, C-4b), 123.8 (2CH, C-3', C-5'), 129.1 (CH, C-7), 130.1 (2CH, C-2', C-6'), 130.7 (C_0, C-4a), 134.6 (C_0, C-9a), 138.0 (C_0, C-1'), 139.7 (C_0, C-3), 141.7 (C_0, C-1), 143.6 (C_0, C-8a), 147.5 (C_0, C-4'), 164.8 (C=O); HRMS-ESI *m/z*, calcd. for $C_{20}H_{16}ClN_4O_3$ $[M + H]^+$: 395.0905, found: 395.0905.

N-(2-Chloroethyl)-1-(4-methoxyphenyl)-9*H*-β-carboline-3-carboxamide (**6h**)

Yield: 82%; mp 169-173 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 3.78 (t, *J* 5.8 Hz, 2H, H-2"), 3.86-3.91 (m, 2H, H-1"), 3.91 (s, 3H, O$\underline{CH_3}$), 7.14 (d, *J* 8.7 Hz, 2H, H-3', H-5'), 7.36 (t, *J* 7.9 Hz, 1H, H-6), 7.55-7.62 (m, 2H, H-7, H-8), 7.95 (d, *J* 8.7 Hz, 2H, H-2', H-6'), 8.20 (d, *J* 7.9 Hz, 1H, H-5), 8.67 (t, *J* 5.9 Hz, CO$\underline{NH}$), 8.84 (s, 1H, H-4), 8.88 (s, 1H, NH, H-9); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 41.3 (CH_2, C-1"), 43.9 (CH_2, C-2"), 55.5 (O$\underline{CH_3}$), 111.8 (CH, C-8), 113.2 (CH, C-4), 114.7 (2CH, C-3', C-5'), 121.0 (CH, C-6), 122.1 (CH, C-5), 122.3 (C_0, C-4b), 128.8 (CH, C-7), 129.5 (2CH, C-2', C-6'), 130.3 (C_0, C-4a), 130.4 (C_0, C-9a), 134.8 (C_0, C-1'), 139.9 (C_0, C-3), 140.7 (C_0, C-1), 141.1 (C_0, C-8a), 160.5 (C_0, C-4'), 165.9 (C=O); HRMS-ESI *m/z*, calcd. for $C_{21}H_{19}ClN_3O_2$ $[M + H]^+$: 380.1160, found: 380.1153.

N-(2-Chloroethyl)-1-[4-(dimethylamino)phenyl]-9*H*-β-carboline-3-carboxamide (**6i**)

Yield: 85%; mp > 304 °C (decomp.); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 3.04 (s, 6H, N($\underline{CH_3}$)$_2$), 3.76 (t, *J* 6.0 Hz, 2H, H-2"), 3.88 (q, *J* 6.0 Hz, 2H, H-1"), 6.90 (d, *J* 8.8 Hz, 2H, H-3', H-5'), 7.33 (ddd, *J* 7.9, 5.3, 2.7 Hz, 1H, H-6), 7.53-7.57 (m, 2H, H-7, H-8), 7.89 (d, *J* 8.8 Hz, 2H, H-2', H-6'), 8.18 (d, *J* 7.9 Hz, 1H, H-5), 8.72 (t, *J* 6.0 Hz, CO$\underline{NH}$), 8.78 (s, 1H, H-4), 8.90 (s, 1H, NH, H-9); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 40.3 (N($\underline{C}H_3$)$_2$), 41.3 (CH_2, C-1"), 43.9 (CH_2, C-2"), 111.7 (CH, C-8), 112.5 (2CH, C-3', C-5'), 112.5 (CH, C-4), 120.8 (CH, C-6), 122.1 (CH, C-5), 122.4 (C_0, C-4b), 125.4 (C_0, C-4a), 128.6 (CH, C-7), 129.0 (2CH, C-2', C-6'), 130.0 (C_0, C-9a), 134.7 (C_0, C-1'), 139.8 (C_0, C-3), 140.6 (C_0, C-1), 141.8 (C_0, C-8a), 151.0 (C_0, C-4'), 166.1 (C=O); HRMS-ESI *m/z*, calcd. for $C_{22}H_{22}ClN_4O$ $[M + H]^+$: 393.1477, found: 393.1465.

N-(3-Chloropropyl)-1-phenyl-9*H*-β-carboline-3-carboxamide (**7a**)

Yield: 82%; mp 161-164 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 2.16 (qt, *J* 6.4 Hz, 2H, H-2"), 3.65-3.70 (m, 4H, H-1", H-3"), 7.34 (t, *J* 7.8 Hz, 1H, H-6), 7.51-7.62 (m, 5H, H-7, H-8, H-3', H-4', H-5'), 7.97 (d, *J* 7.3 Hz, 2H, H-2', H-6'), 8.18 (d, *J* 7.8 Hz, 1H, H-5), 8.42 (s, 1H, CO$\underline{NH}$), 8.87 (s, H-4), 8.97 (s, 1H, NH, H-9); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 32.6 (CH_2, C-2"), 37.0 (CH_2, C-1"), 42.7 (CH_2, C-3"), 111.8 (CH, C-8), 113.5 (CH, C-4), 121.0 (CH, C-6), 122.2 (CH, C-5), 122.3 (C_0, C-4b), 128.2 (2CH, C-2', C-6'), 128.9 (CH, C-4'), 129.3 (CH, C-7), 129.3 (CH, C-3', C-5'), 130.6 (C_0, C-4a), 134.8 (C_0, C-9a), 137.9 (C_0, C-1'), 140.3 (C_0, C-3), 140.7 (C_0, C-1), 141.1 (C_0, C-8a), 165.9 (C=O); HRMS-ESI *m/z*, calcd. for $C_{21}H_{19}ClN_3O$ $[M + H]^+$: 364.1211, found: 364.1181.

N-(3-Chloropropyl)-1-(2-fluorophenyl)-9*H*-β-carboline-3-carboxamide (**7b**)

Yield: 52%; mp 173-174 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 2.17 (qt, *J* 6.6 Hz, 2H, H-2"), 3.65-3.73 (m, 4H, H-1", H-3"), 7.28-7.43 (m, 3H, H-6, H-3', H-6'), 7.50-7.62 (m, 3H, H-4', H-7, H-8), 7.88 (td, *J* 7.6, 1.8 Hz, 1H, H-5'), 8.22 (d, *J* 7.9 Hz, 1H, H-5), 8.36 (t, *J* 6.1 Hz, 1H, CO$\underline{NH}$), 8.64 (s, 1H, NH, H-9), 8.94 (s, 1H, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 32.6 (CH_2, C-2"), 37.0 (CH_2, C-1"), 42.7 (CH_2, C-3"), 111.8 (CH, C-8), 114.0 (CH, C-4), 116.3 (CH, d, *J* 21.5 Hz, C-3'), 121.0 (CH, C-6), 122.0 (C_0, C-4b), 122.2 (CH, C-5), 125.2 (CH, C-6'), 125.3 (C_0, C-4a), 129.1 (CH, C-7), 130.6 (C_0, C-1'), 131.1 (CH, d, *J* 7.5 Hz, C-4'), 132.3 (CH, d, *J* 3.8 Hz, C-5'), 135.5 (C_0, C-9a), 136.2 (C_0, C-3), 140.4 (C_0, C-1), 140.6 (C_0, C-8a), 159.9 (C_0, d, *J* 246 Hz, 1C, C-2'), 165.7 (C=O); HRMS-ESI *m/z*, calcd. for $C_{21}H_{18}ClFN_3O$ $[M + H]^+$: 382.1117, found: 382.1085.

N-(3-Chloropropyl)-1-(4-fluorophenyl)-9*H*-β-carboline-3-carboxamide (**7c**)

Yield: 92%; mp 131-133 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 2.17 (qt, *J* 6.5 Hz, 2H, H-2"), 3.65-3.73 (m, 4H, H-1", H-3"), 7.28-7.39 (m, 3H, H-6, H-3', H-5'), 7.55-7.62 (m, 2H, H-7, H-8), 7.97 (dd, *J* 8.5, 5.3 Hz, 2H, H-2', H-6'), 8.19 (d, *J* 8.0 Hz, 1H, H-5), 8.38 (t, *J* 6.0 Hz, CO$\underline{NH}$), 8.83 (s, 1H, NH, H-9), 8.87 (s, 1H, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 32.6 (CH_2, C-2"), 37.0 (CH_2, C-1"), 42.7 (CH_2, C-3"), 111.8 (CH, C-8), 113.6 (CH, C-4), 116.4 (d, *J* 21.3 Hz, 2CH, C-3', C-5'), 121.2 (CH, C-6), 122.2 (CH, C-5), 122.3 (C_0, C-4b), 129.1 (CH, C-7), 130.1 (d, *J* 8.5 Hz, 2CH, C-2', C-6'), 130.8 (C_0, C-4a), 134.0 (C_0, C-1'), 134.7 (C_0, C-9a), 140.1 (C_0, C-3), 140.4 (C_0, C-1), 140.7 (C_0, C-8a), 163.3 (d, *J* 247.7 Hz, C_0, C-4'), 165.8 (C=O); HRMS-ESI *m/z*, calcd. for $C_{21}H_{18}ClFN_3O$ $[M + H]^+$: 382.1117, found: 382.1091.

N-(3-Chloropropyl)-1-(2-chlorophenyl)-9*H*-β-carboline-3-carboxamide (**7d**)

Yield: 95%; mp 195-198 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 2.14 (qt, *J* 6.5 Hz, 2H, H-2"), 3.62-3.68 (m, 4H, H-1", H-3"), 7.35 (t, *J* 7.8 Hz, 1H, H-6), 7.46-7.65 (m, 6H, H-7, H-8, H-3', H-4', H-5', H-6'), 8.21 (d, *J* 7.8 Hz, 1H, H-5), 8.31 (t, *J* 5.5 Hz, 1H, CO$\underline{NH}$), 8.63 (s, 1H, NH, H-9), 8.92 (s, 1H, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 32.6 (CH_2, C-2"), 36.9 (CH_2, C-1"), 42.6 (CH_2, C-3"), 111.8 (CH, C-8), 114.1 (CH, C-4), 121.0 (CH, C-6), 122.1 (C_0, C-4b), 122.2 (CH, C-5), 127.4 (CH, C-7), 129.1 (CH, C-5'), 130.2 (C_0, C-4a), 130.4 (CH, C-3'), 130.5 (CH, C-6'), 131.9 (CH, C-4'), 133.0 (C_0, C-2'), 135.4 (C_0, C-9a), 136.3 (C_0, C-1'), 139.4 (C_0, C-3), 140.0 (C_0, C-1), 140.8 (C_0, C-8a), 165.8

(C=O); HRMS-ESI *m/z*, calcd. for $C_{21}H_{18}Cl_2N_3O$ $[M + H]^+$: 398.0821, found: 398.0782.

N-(3-Chloropropyl)-1-(4-chlorophenyl)-9*H*-β-carboline-3-carboxamide (**7e**)

Yield: 93%; mp 175-178 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 2.17 (qt, *J* 6.4 Hz, 2H, H-2"), 3.66-3.72 (m, 4H, H-1", H-3"), 7.36 (t, *J* 7.8 Hz, 1H, H-6), 7.56-7.58 (m, 4H, H-7, H-8, H-3', H-5'), 7.92 (d, *J* 7.8 Hz, 2H, H-2', H-6'), 8.17 (d, *J* 7.8 Hz, 1H, H-5), 8.37 (s, 1H, CONH), 8.86 (s, 1H, H-4), 8.91 (s, 1H, NH, H-9); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 32.5 (CH_2, C-2"), 37.0 (CH_2, C-1"), 42.7 (CH_2, C-3"), 111.9 (CH, C-8), 113.7 (CH, C-4), 121.2 (CH, C-6), 122.2 (CH, C-5), 122.2 (C_0, C-4b), 129.1 (CH, C-7), 129.5 (4CH, C-3', C-5', C-2', C-6'), 130.9 (C_0, C-4a), 134.7 (C_0, C-4'), 135.3 (C_0, C-9a), 136.3 (C_0, C-1'), 139.8 (C_0, C-3), 140.4 (C_0, C-1), 140.8 (C_0, C-8a), 165.7 (C=O); HRMS-ESI *m/z*, calcd. for $C_{21}H_{18}Cl_2N_3O$ $[M + H]^+$: 398.0821, found: 398.0791.

N-(3-Chloropropyl)-1-(3-nitrophenyl)-9*H*-β-carboline-3-carboxamide (**7f**)

Yield: 93%; mp 203-206 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 2.07 (qt, *J* 6.5 Hz, 2H, H-2"), 3.53 (q, *J* 6.5 Hz, 2H, H-1"), 3.73 (t, *J* 6.5 Hz, 2H, H-3"), 7.34 (t, *J* 7.2 Hz, 1H, H-6), 7.60-7.70 (m, 2H, H-7, H-8), 7.94 (t, *J* 7.9 Hz, 1H, H-5'), 8.40-8.46 (m, 2H, H-5, H-4'), 8.60 (d, *J* 7.9 Hz, 1H, H-6'), 8.86-8.92 (m, 3H, H-2', H-4, CONH), 12.03 (s, 1H, NH, H-9); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 32.5 (CH_2, C-2"), 36.7 (CH_2, C-1"), 43.4 (CH_2, C-3"), 112.5 (CH, C-8), 113.9 (CH, C-4), 120.4 (CH, C-6), 121.2 (C_0, C-4b), 122.2 (CH, C-5), 123.5 (CH, C-2'), 123.5 (CH, C-4'), 128.9 (CH, C-7), 130.3 (CH, C-5'), 130.4 (C_0, C-4a), 134.4 (C_0, C-9a), 135.4 (CH, C-6'), 138.2 (C_0, C-1'), 138.9 (C_0, C-3), 140.1 (C_0, C-1), 141.6 (C_0, C-8a), 148.3 (C_0, C-3'), 164.8 (C=O); HRMS-ESI *m/z*, calcd. for $C_{21}H_{18}ClN_4O_3$ $[M + H]^+$: 409.1062, found: 409.1032.

N-(3-Chloropropyl)-1-(4-nitrophenyl)-9*H*-β-carboline-3-carboxamide (**7g**)

Yield: 83%; mp 233-236 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 2.08 (qt, *J* 6.6 Hz, 2H, H-2"), 3.53 (q, *J* 6.6 Hz, 2H, H-1"), 3.73 (t, *J* 6.6 Hz, 2H, H-3"), 7.34 (t, *J* 7.1 Hz, 1H, H-6), 7.60-7.71 (m, 2H, H-7, H-8), 8.43-8.50 (m, 5H, H-5, H-2', H-3', H-5', H-6'), 8.88-8.91 (m, 2H, H-4, CONH), 12.03 (s, 1H, NH, H-9); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 32.6 (CH_2, C-2"), 36.7 (CH_2, C-1"), 43.3 (CH_2, C-3"), 112.6 (CH, C-8), 114.1 (CH, C-4), 120.5 (CH, C-6), 121.1 (C_0, C-4b), 122.2 (CH, C-5), 123.7 (2CH, C-3', C-5'), 129.0 (CH, C-7), 130.1 (2CH, C-2', C-6'), 130.7 (C_0, C-4a), 134.5 (C_0, C-9a), 137.9 (C_0, C-1'), 140.1 (C_0, C-3), 141.7 (C_0, C-1), 143.7 (C_0, C-8a), 147.4 (C_0, C-4'), 164.8 (C=O); HRMS-ESI *m/z*, calcd. for $C_{21}H_{18}ClN_4O_3$ $[M + H]^+$: 409.1062, found: 409.1040.

N-(3-Chloropropyl)-1-(4-methoxyphenyl)-9*H*-β-carboline-3-carboxamide (**7h**)

Yield: 85%; mp 192-194 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 2.17 (qt, *J* 6.5 Hz, 2H, H-2"), 3.66-3.71 (m, 4H, H-1", H-3"), 3.90 (s, 3H, OCH_3), 7.13 (d, *J* 8.4 Hz, 2H, H-3', H-5'), 7.35 (t, *J* 7.9 Hz, 1H, H-6), 7.55-7.59 (m, 2H, H-7, H-8), 7.92 (d, *J* 8.4 Hz, 2H, H-2', H-6'), 8.19 (d, *J* 7.9 Hz, 1H, H-5), 8.42 (t, *J* 6.0 Hz, CONH), 8.84 (s, 1H, H-4), 8.87 (s, 1H, NH, H-9); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 32.6 (CH_2, C-2"), 37.0 (CH_2, C-1"), 42.7 (CH_2, C-3"), 55.5 (OCH_3), 111.8 (CH, C-8), 113.1 (CH, C-4), 114.7 (2CH, C-3', C-5'), 121.0 (CH, C-6), 122.2 (CH, C-5), 122.4 (C_0, C-4b), 128.8 (CH, C-7), 129.5 (2CH, C-2', C-6'), 130.3 (C_0, C-4a), 130.4 (C_0, C-9a), 134.7 (C_0, C-1'), 140.3 (C_0, C-3), 140.7 (C_0, C-1), 141.0 (C_0, C-8a), 160.5 (C_0, C-4'), 165.9 (C=O); HRMS-ESI *m/z*, calcd. for $C_{22}H_{21}ClN_3O_2$ $[M + H]^+$: 394.1317, found: 394.1338.

N-(3-Chloropropyl)-1-[4-(dimethylamino)phenyl]-9*H*-β-carboline-3-carboxamide (**7i**)

Yield: 62%; mp 198-202 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 2.14 (qt, *J* 6.6 Hz, 2H, H-2"), 2.99 (s, 6H, N(CH_3)$_2$), 3.63-3.70 (m, 4H, H-1", H-3"), 6.85 (d, *J* 8.8 Hz, 2H, H-3', H-5'), 7.31 (td, *J* 7.9, 1.3 Hz, 1H, H-6), 7.51-7.59 (m, 2H, H-7, H-8), 7.88 (d, *J* 8.8 Hz, 2H, H-2', H-6'), 8.14 (d, *J* 7.9 Hz, 1H, H-5), 8.48 (t, *J* 6.2 Hz, CONH), 8.76 (s, 1H, H-4), 9.16 (s, 1H, NH, H-9); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 32.6 (CH_2, C-2"), 36.9 (CH_2, C-1"), 40.2 (N(CH_3)$_2$), 42.7 (CH_2, C-3"), 111.8 (CH, C-8), 112.3 (2CH, C-3', C-5'), 112.4 (CH, C-4), 120.6 (CH, C-6), 122.0 (CH, C-5), 122.3 (C_0, C-4b), 125.3 (C_0, C-4a), 128.4 (CH, C-7), 129.0 (2CH, C-2', C-6'), 130.0 (C_0, C-9a), 134.6 (C_0, C-1'), 139.9 (C_0, C-3), 140.7 (C_0, C-1), 141.7 (C_0, C-8a), 150.9 (C_0, C-4'), 166.1 (C=O); HRMS-ESI *m/z*, calcd. for $C_{23}H_{24}ClN_4O$ $[M + H]^+$: 407.1633, found: 407.1650.

Synthesis of 1-(substituted-phenyl)-3-(4,5-dihydro-1,3-oxazol-2-yl)-9*H*-β-carboline (**8a-8i**)

To a solution of intermediates 2-chloroethyl-β-carboline-3-carboxamides **6a-6i** (0.3 mmol) in DMF (3 mL), it was added 2 equivalents of potassium carbonate (0.08 g). The reaction mixture was refluxed until complete consumption of intermediates (14-20 h). After this time, the solution was cooled, treated with 5 mL of distilled water and left on ice bath for 3 h. The solid formed was filtered under vacuum, washed with distilled water, and recrystallized

with methanol. The derivatives **8a**-**8i** were obtained in yields in the range of 46-93%.

1-Phenyl-3-(4,5-dihydro-1,3-oxazol-2-yl)-9*H*-β-carboline (**8a**)

Yield: 85%; mp 224-226 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 4.18 (t, *J* 9.3 Hz, 2H, H-4"), 4.59 (t, *J* 9.3 Hz, 2H, H-5"), 7.35 (t, *J* 7.9 Hz, 1H, H-6), 7.44-7.61 (m, 5H, H-7, H-8, H-3', H-4', H-5'), 7.96 (d, *J* 7.2 Hz, 2H, H-2', H-6'), 8.18 (d, *J* 7.9 Hz, 1H, H-5), 8.76 (s, H-4), 8.82 (s, 1H, NH, H-9); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 55.0 (CH_2, C-4"), 68.2 (CH_2, C-5"), 111.8 (CH, C-8), 115.1 (CH, C-4), 120.9 (CH, C-6), 122.0 (CH, C-5), 122.1 (C_0, C-4b), 128.4 (2CH, C-2', C-6'), 128.8 (CH, C-4'), 129.0 (CH, C-7), 129.1 (CH, C-3', C-5'), 129.9 (C_0, C-4a), 134.4 (C_0, C-9a), 136.9 (C_0, C-3), 138.0 (C_0, C-1'), 140.6 (C_0, C-8a), 142.9 (C_0, C-1), 165.0 (C_0, C-2"); HRMS-ESI *m/z*, calcd. for $C_{20}H_{16}N_3O$ $[M + H]^+$: 314.1288, found: 314.1245.

1-(2-Fluorophenyl)-3-(4,5-dihydro-1,3-oxazol-2-yl)-9*H*-β-carboline (**8b**)

Yield: 46%; mp 261-263 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 4.17 (t, *J* 9.5 Hz, 2H, H-4"), 4.58 (t, *J* 9.5 Hz, 2H, H-5"), 7.20-7.36 (m, 3H, H-6, H-3', H-6'), 7.42-7.60 (m, 3H, H-4', H-7, H-8), 7.87 (td, *J* 7.5, 1.4 Hz, 1H, H-5'), 8.17 (d, *J* 7.8 Hz, 1H, H-5), 8.69 (s, 1H, NH, H-9), 8.80 (s, 1H, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 55.0 (CH_2, C-4"), 68.2 (CH_2, C-5"), 111.8 (CH, C-8), 115.5 (CH, C-4), 116.3 (CH, d, *J* 21.9 Hz, C-3'), 120.8 (CH, C-6), 121.8 (C_0, C-4b), 121.9 (CH, C-5), 125.1 (CH, C-6'), 125.3 (C_0, C-4a), 129.0 (CH, C-7), 129.8 (C_0, C-9a), 130.9 (CH, d, *J* 7.9 Hz, C-4'), 132.7 (CH, C-5'), 135.2 (C_0, C-3), 136.8 (C_0, C-1'), 138.0 (C_0, C-8a), 140.6 (C_0, C-1), 159.9 (C_0, d, *J* 245 Hz, 1C, C-2'), 164.9 (C_0, C-2"); HRMS-ESI *m/z*, calcd. for $C_{20}H_{15}FN_3O$ $[M + H]^+$: 332.1194, found: 332.1166.

1-(4-Fluorophenyl)-3-(4,5-dihydro-1,3-oxazol-2-yl)-9*H*-β-carboline (**8c**)

Yield: 51%; mp > 246 °C (decomp.); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 4.15 (t, *J* 9.5 Hz, 2H, H-4"), 4.57 (t, 9.5 Hz, 2H, H-5"), 7.08 (t, *J* 8.8 Hz, 2H, H-3', H-5'), 7.33 (ddd, *J* 7.9, 5.7, 2.3 Hz, 1H, H-6), 7.53-7.56 (m, 2H, H-7, H-8), 7.84 (dd, *J* 8.8, 5.4 Hz, 2H, H-2', H-6'), 8.14 (d, *J* 7.9 Hz, 1H, H-5), 8.70 (s, 1H, H-4), 9.16 (s, 1H, NH, H-9); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 54.9 (CH_2, C-4"), 68.2 (CH_2, C-5"), 111.9 (CH, C-8), 115.1 (CH, C-4), 115.9 (d, *J* 21.9 Hz, 2CH, C-3', C-5'), 120.9 (CH, C-6), 121.9 (CH, C-5), 122.0 (C_0, C-4b), 128.9 (CH, C-7), 129.9 (C_0, C-4a), 130.2 (d, *J* 8.5 Hz, 2CH, C-2', C-6'), 134.0 (C_0, C-9a), 134.3 (C_0, C-3), 136.6 (C_0, C-1'), 140.8 (C_0, C-8a), 141.9 (C_0, C-1), 163.1 (d, *J* 247.2 Hz, C_0, C-4'), 165.0 (C_0, C-2"); HRMS-ESI *m/z*, calcd. for $C_{20}H_{15}FN_3O$ $[M + H]^+$: 332.1194, found: 332.1149.

1-(2-Chlorophenyl)-3-(4,5-dihydro-1,3-oxazol-2-yl)-9*H*-β-carboline (**8d**)

Yield: 65%; mp 246-249 °C; ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD) δ_H 4.14 (t, *J* 9.6 Hz, 2H, H-4"), 4.58 (t, *J* 9.6 Hz, 2H, H-5"), 7.32 (m, 1H, H-6), 7.38-7.45 (m, 2H, H-7, H-6'), 7.51-7.61 (m, 4H, H-8, H-3', H-4', H-5'), 8.20 (d, *J* 7.9 Hz, 1H, H-5), 8.80 (s, 1H, H-4); ^{13}C NMR (125 MHz, $CDCl_3$/CD_3OD) δ_C 54.5 (CH_2, C-4"), 68.4 (CH_2, C-5"), 112.1 (CH, C-8), 115.9 (CH, C-4), 120.7 (CH, C-6), 121.7 (C_0, C-4b), 122.0 (CH, C-5), 127.3 (CH, C-7), 129.0 (CH, C-5'), 129.4 (C_0, C-4a), 129.8 (CH, C-3'), 130.4 (CH, C-6'), 132.3 (CH, C-4'), 133.4 (C_0, C-2'), 135.4 (C_0, C-9a), 135.6 (C_0, C-3), 136.7 (C_0, C-1'), 141.2 (C_0, C-8a), 141.4 (C_0, C-1), 165.6 (C_0, C-2"); HRMS-ESI *m/z*, calcd. for $C_{20}H_{15}ClN_3O$ $[M + H]^+$: 348.0898, found: 348.0854.

1-(4-Chlorophenyl)-3-(4,5-dihydro-1,3-oxazol-2-yl)-9*H*-β-carboline (**8e**)

Yield: 50%; mp 278-282 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 4.19 (t, *J* 9.5 Hz, 2H, H-4"), 4.59 (t, *J* 9.5 Hz, 2H, H-5"), 7.36 (t, *J* 7.9 Hz, 1H, H-6), 7.51-7.62 (m, 4H, H-7, H-8, H-3', H-5'), 7.91 (d, *J* 8.2 Hz, 2H, H-2', H-6'), 8.18 (d, *J* 7.9 Hz, 1H, H-5), 8.68 (s, 1H, NH, H-9), 8.75 (s, 1H, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 55.1 (CH_2, C-4"), 68.3 (CH_2, C-5"), 111.8 (CH, C-8), 115.3 (CH, C-4), 121.1 (CH, C-6), 122.0 (CH, C-5), 122.0 (C_0, C-4b), 129.1 (CH, C-7), 129.4 (2CH, C-3', C-5'), 129.7 (2CH, C-2', C-6'), 130.2 (C_0, C-4a), 134.3 (C_0, C-4'), 135.1 (C_0, C-9a), 136.4 (C_0, C-3), 137.1 (C_0, C-1'), 140.6 (C_0, C-8a), 141.6 (C_0, C-1), 164.8 (C_0, C-2"); HRMS-ESI *m/z*, calcd. for $C_{20}H_{15}ClN_3O$ $[M + H]^+$: 348.0898, found: 348.0906.

1-(3-Nitrophenyl)-3-(4,5-dihydro-1,3-oxazol-2-yl)-9*H*-β-carboline (**8f**)

Yield: 58%; mp > 250 °C (decomp.); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 4.06 (t, *J* 9.5 Hz, 2H, H-4"), 4.50 (t, *J* 9.5 Hz, 2H, H-5"), 7.34 (t, *J* 7.9 Hz, 1H, H-6), 7.61-7.69 (m, 2H, H-7, H-8), 7.93 (t, *J* 7.9 Hz, 1H, H-5'), 8.39-8.44 (m, 2H, H-5, H-4'), 8.48 (d, *J* 7.9 Hz, 1H, H-6'), 8.79 (t, *J* 1.8 Hz, 1H, H-2'), 8.87 (s, 1H, H-4), 12.01 (s, 1H, NH, H-9); ^{13}C NMR (125 MHz, DMSO-d_6) δ_C 54.4 (CH_2, C-4"), 67.4 (CH_2, C-5"), 112.5 (CH, C-8), 115.6 (CH, C-4), 120.3 (CH, C-6), 120.8 (C_0, C-4b), 122.1 (CH, C-5), 123.2 (CH, C-2'), 123.4 (CH, C-4'), 128.9 (CH, C-7), 129.9 (C_0, C-4a), 130.3 (CH, C-5'), 133.9 (C_0, C-9a), 134.8 (CH, C-6'), 135.9 (C_0, C-3), 139.0 (C_0, C-1'), 139.0 (C_0, C-8a), 141.5 (C_0, C-1), 148.1 (C_0, C-3'), 163.4 (C_0, C-2"); HRMS-ESI *m/z*, calcd. for $C_{20}H_{15}N_4O_3$ $[M + H]^+$: 359.1139, found: 359.1098.

1-(4-Nitrophenyl)-3-(4,5-dihydro-1,3-oxazol-2-yl)-9*H*-β-carboline (**8g**)

Yield: 74%; mp 323-326 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 4.06 (t, *J* 9.5 Hz, 2H, H-4"), 4.50 (t, *J* 9.5 Hz, 2H, H-5"), 7.34 (ddd, *J* 7.9, 7.0, 1.1 Hz, 1H, H-6), 7.63 (ddd, *J* 8.2, 7.0, 1.1 Hz, 1H, H-7), 7.69 (d, *J* 8.2 Hz, 1H, H-8), 8.32 (d, *J* 9.0 Hz, 2H, H-2', H-6'), 8.43 (d, *J* 7.9 Hz, 1H, H-5), 8.47 (d, *J* 9.0 Hz, 2H, H-3', H-5'), 8.88 (s, 1H, H-4), 12.01 (s, 1H, NH, H-9); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 54.5 (CH_2, C-4"), 67.5 (CH_2, C-5"), 112.6 (CH, C-8), 115.9 (CH, C-4), 120.5 (CH, C-6), 120.9 (C_0, C-4b), 122.2 (CH, C-5), 123.9 (2CH, C-3', C-5'), 129.0 (CH, C-7), 129.8 (2CH, C-2', C-6'), 130.1 (C_0, C-4a), 134.1 (C_0, C-9a), 136.1 (C_0, C-3), 139.0 (C_0, C-1'), 141.6 (C_0, C-8a), 143.9 (C_0, C-1), 147.4 (C_0, C-4'), 163.5 (C_0, C-2"); HRMS-ESI *m/z*, calcd. for $C_{20}H_{15}N_4O_3$ $[M + H]^+$: 359.1139, found: 359.1101.

1-(4-Methoxyphenyl)-3-(4,5-dihydro-1,3-oxazol-2-yl)-9*H*-β-carboline (**8h**)

Yield: 81%; mp 247-250 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 3.84 (s, 3H, O$\underline{C}H_3$), 4.16 (t, *J* 9.5 Hz, 2H, H-4"), 4.57 (t, *J* 9.5 Hz, 2H, H-5"), 6.98 (d, *J* 8.4 Hz, 2H, H-3', H-5'), 7.33 (t, *J* 7.8 Hz, 1H, H-6), 7.52-7.57 (m, 2H, H-7, H-8), 7.86 (d, *J* 8.4 Hz, 2H, H-2', H-6'), 8.15 (d, *J* 7.8 Hz, 1H, H-5), 8.69 (s, 1H, H-4), 8.95 (s, 1H, NH, H-9); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 55.0 (CH_2, C-4"), 55.4 (O$\underline{C}H_3$), 68.2 (CH_2, C-5"), 111.8 (CH, C-8), 114.4 (2CH, C-3', C-5'), 114.6 (CH, C-4), 120.8 (CH, C-6), 121.9 (CH, C-5), 122.1 (C_0, C-4b), 128.7 (CH, C-7), 129.6 (2CH, C-2', C-6'), 130.5 (C_0, C-4a), 134.3 (C_0, C-9a), 136.7 (C_0, C-3), 136.7 (C_0, C-1'), 140.7 (C_0, C-8a), 142.8 (C_0, C-1), 160.2 (C_0, C-4'), 165.1 (C_0, C-2"); HRMS-ESI *m/z*, calcd. for $C_{21}H_{18}N_3O_2$ $[M + H]^+$: 344.1394, found: 344.1396.

1-(4-(Dimethylamino)phenyl)-3-(4,5-dihydro-1,3-oxazol-2-yl)-9*H*-β-carboline (**8i**)

Yield: 93%; mp 235-238 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 2.96 (s, 6H, N($\underline{C}H_3$)$_2$), 4.16 (t, *J* 9.5 Hz, 2H, H-4"), 4.56 (t, *J* 9.5 Hz, 2H, H-5"), 6.72 (d, *J* 8.7 Hz, 2H, H-3', H-5'), 7.30 (dt, *J* 8.0, 4.0 Hz, 1H, H-6), 7.52-7.53 (m, 2H, H-7, H-8), 7.81 (d, *J* 8.7 Hz, 2H, H-2', H-6'), 8.13 (d, *J* 8.0 Hz, 1H, H-5), 8.63 (s, 1H, H-4), 9.03 (s, 1H, NH, H-9); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 40.3 (N($\underline{C}H_3$)$_2$), 55.0 (CH_2, C-4"), 68.1 (CH_2, C-5"), 111.8 (CH, C-8), 112.3 (2CH, C-3', C-5'), 113.9 (CH, C-4), 120.5 (CH, C-6), 121.8 (CH, C-5), 122.2 (C_0, C-4b), 125.7 (C_0, C-4a), 128.4 (CH, C-7), 129.2 (2CH, C-2', C-6'), 129.3 (C_0, C-9a), 134.3 (C_0, C-3), 136.5 (C_0, C-1'), 140.6 (C_0, C-8a), 143.6 (C_0, C-1), 150.8 (C_0, C-4'), 165.3 (C_0, C-2"); HRMS-ESI *m/z*, calcd. for $C_{22}H_{21}N_4O$ $[M + H]^+$: 357.1710, found: 357.1712.

Synthesis of 1-(substituted-phenyl)-3-(5,6-dihydro-4*H*-1,3-oxazin-2-yl)-β-carboline (**9a**-**9h**)

To a solution of intermediates 3-chloropropyl-β-carboline-3-carboxamides **7a**-**7h** (0.3 mmol) in DMF (3 mL), it was added 2 equivalents of potassium carbonate (0.08 g). The reaction mixture was irradiated in a microwave oven at 100% power level for 5-9 min. After all the starting material was consumed (5-9 min), the reaction mixture was poured into water and the precipitate formed was filtered under vacuum, washed with water and recrystallized with acetonitrile. The derivatives **9a**-**9h** were obtained in yields in the range of 45-86%.

1-Phenyl-3-(5,6-dihydro-4*H*-1,3-oxazin-2-yl)-9*H*-β-carboline (**9a**)

Yield: 45%; mp 231-234 °C; ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD) δ_H 2.04 (qt, *J* 5.4 Hz, 2H, H-5"), 3.68 (t, *J* 5.4 Hz, 2H, H-4"), 4.47 (t, *J* 5.4 Hz, 2H, H-6"), 7.25-7.31 (m, 1H, H-6), 7.36-7.54 (m, 5H, H-7, H-8, H-3', H-4', H-5'), 7.91 (d, *J* 7.0 Hz, 2H, H-2', H-6'), 8.14 (d, *J* 7.8 Hz, 1H, H-5), 8.61 (s, 1H, H-4); ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD) δ_C 21.9 (CH_2, C-5"), 42.8 (CH_2, C-4"), 65.6 (CH_2, C-6"), 111.8 (CH, C-8), 113.4 (CH, C-4), 120.4 (CH, C-6), 121.9 (CH, C-5), 122.1 (C_0, C-4b), 128.5 (2CH, C-2', C-6'), 128.5 (CH, C-4'), 128.8 (CH, C-7), 128.9 (CH, C-3', C-5'), 130.0 (C_0, C-4a), 134.3 (C_0, C-9a), 138.3 (C_0, C-1'), 140.9 (C_0, C-8a), 141.6 (C_0, C-3), 142.3 (C_0, C-1), 156.3 (C_0, C-2"); HRMS-ESI *m/z*, calcd. for $C_{21}H_{18}N_3O$ $[M + H]^+$: 328.1444, found: 328.1476.

1-(2-Fluorophenyl)-3-(5,6-dihydro-4*H*-1,3-oxazin-2-yl)-9*H*-β-carboline (**9b**)

Yield: 73%; mp 234-239 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 2.07 (qt, *J* 5.6 Hz, 2H, H-5"), 3.74 (t, *J* 5.6 Hz, 2H, H-4"), 4.51 (t, *J* 5.6 Hz, 2H, H-6"), 7.21-7.35 (m, 3H, H-6, H-3', H-6'), 7.43-7.59 (m, 3H, H-4', H-7, H-8), 7.91 (td, *J* 7.5, 1.6 Hz, 1H, H-5'), 8.19 (d, *J* 7.8 Hz, 1H, H-5), 8.46 (s, 1H, NH, H-9), 8.74 (s, 1H, H-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 22.0 (CH_2, C-5"), 43.0 (CH_2, C-4"), 65.7 (CH_2, C-6"), 111.7 (CH, C-8), 113.9 (CH, C-4), 115.8 (CH, d, *J* 21.9 Hz, C-3'), 120.6 (CH, C-6), 122.0 (CH, C-5), 122.1 (C_0, C-4b), 125.1 (CH, C-6'), 125.2 (C_0, C-4a), 128.8 (CH, C-7), 130.1 (C_0, C-9a), 130.7 (CH, d, *J* 8.5 Hz, C-4'), 132.9 (CH, d, *J* 4.0 Hz, C-5'), 135.0 (C_0, C-1'), 137.2 (C_0, C-8a), 140.7 (C_0, C-3), 142.2 (C_0, C-1), 155.7 (C_0, C-2"), 160.0 (C_0, d, *J* 244.9 Hz, 1C, C-2'); HRMS-ESI *m/z*, calcd. for $C_{21}H_{17}FN_3O$ $[M + H]^+$: 346.1350, found: 346.1345.

1-(4-Fluorophenyl)-3-(5,6-dihydro-4*H*-1,3-oxazin-2-yl)-9*H*-β-carboline (**9c**)

Yield: 45%; mp 228-231 °C; ^{1}H NMR (300 MHz,

$CDCl_3/CD_3OD$) δ_H 2.06 (dt, *J* 5.7 Hz, 2H, H-5"), 3.70 (t, *J* 5.7 Hz, 2H, H-4"), 4.49 (t, *J* 5.7 Hz, 2H, H-6"), 7.14 (t, *J* 8.7 Hz, 2H, H-3', H-5'), 7.28-7.33 (m, 1H, H-6), 7.49-7.57 (m, 2H, H-7, H-8), 7.91 (dd, *J* 8.7, 5.5 Hz, 2H, H-2', H-6'), 8.15 (d, *J* 7.9 Hz, 1H, H-5), 8.57 (s, 1H, H-4); ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$) δ_C 21.9 (CH_2, C-5"), 42.7 (CH_2, C-4"), 65.6 (CH_2, C-6"), 111.9 (CH, C-8), 113.4 (CH, C-4), 115.8 (d, *J* 21.3 Hz, 2CH, C-3', C-5'), 120.5 (CH, C-6), 121.8 (CH, C-5), 122.1 (C_0, C-4b), 128.6 (CH, C-7), 130.1 (C_0, C-4a), 130.4 (d, *J* 8.5 Hz, 2CH, C-2', C-6'), 134.1 (C_0, C-9a), 134.4 (C_0, C-1'), 141.0 (C_0, C-8a), 141.2 (C_0, C-3), 141.4 (C_0, C-1), 156.4 (C_0, C-2"), 163.1 (d, *J* 246.6 Hz, C_0, C-4'); HRMS-ESI *m/z*, calcd. for $C_{21}H_{17}FN_3O$ $[M + H]^+$: 346.1350, found: 346.1386.

1-(2-Chlorophenyl)-3-(5,6-dihydro-4*H*-1,3-oxazin-2-yl)-9*H*-β-carboline (**9d**)

Yield: 86%; mp 236-238 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 1.93 (qt, *J* 5.5 Hz, 2H, H-5"), 3.55 (t, *J* 5.5 Hz, 2H, H-4"), 4.36 (t, *J* 5.5 Hz, 2H, H-6"), 7.27 (dt, *J* 7.8, 4.0 Hz, 1H, H-6), 7.51-7.70 (m, 6H, H-7, H-8, H-3', H-4', H-5', H-6'), 8.35 (d, *J* 7.8 Hz, 1H, H-5), 8.75 (s, 1H, H-4), 11.48 (s, 1H, NH, H-9); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 21.6 (CH_2, C-5"), 42.1 (CH_2, C-4"), 64.7 (CH_2, C-6"), 112.2 (CH, C-8), 113.6 (CH, C-4), 119.8 (CH, C-6), 121.1 (C_0, C-4b), 122.0 (CH, C-5), 127.4 (CH, C-7), 128.5 (CH, C-5'), 128.6 (C_0, C-4a), 129.6 (CH, C-3'), 130.4 (CH, C-6'), 132.0 (CH, C-4'), 132.6 (C_0, C-2'), 134.5 (C_0, C-9a), 136.9 (C_0, C-1'), 140.0 (C_0, C-8a), 140.7 (C_0, C-3), 141.4 (C_0, C-1), 154.7 (C_0, C-2"); HRMS-ESI *m/z*, calcd. for $C_{21}H_{17}ClN_3O$ $[M + H]^+$: 362.1055, found: 362.1036.

1-(4-Chlorophenyl)-3-(5,6-dihydro-4*H*-1,3-oxazine)-9*H*-β-carboline (**9e**)

Yield: 60%; mp 234-236 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 1.95 (qt, *J* 5.4 Hz, 2H, H-5"), 3.57 (t, *J* 5.4 Hz, 2H, H-4"), 4.40 (t, *J* 5.4 Hz, 2H, H-6"), 7.28 (t, *J* 7.9 Hz, 1H, H-6), 7.55-7.63 (m, 2H, H-7, H-8), 7.68 (d, *J* 8.4 Hz, 2H, H-3', H-5'), 8.04 (d, *J* 8.4 Hz, 2H, H-2', H-6'), 8.35 (d, *J* 7.9 Hz, 1H, H-5), 8.70 (s, 1H, H-4), 11.69 (s, 1H, NH, H-9); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 21.6 (CH_2, C-5"), 42.1 (CH_2, C-4"), 64.8 (CH_2, C-6"), 112.5 (CH, C-8), 113.4 (CH, C-4), 120.0 (CH, C-6), 121.2 (CH, C-4b), 121.9 (C_0, C-5), 128.5 (CH, C-7), 128.7 (2CH, C-3', C-5'), 129.8 (C_0, C-4a), 130.3 (2CH, C-2', C-6'), 133.4 (C_0, C-4'), 133.5 (C_0, C-9a), 136.8 (C_0, C-1'), 139.4 (C_0, C-8a), 141.2 (C_0, C-3), 141.6 (C_0, C-1), 154.7 (C_0, C-2"); HRMS-ESI *m/z*, calcd. for $C_{21}H_{17}ClN_3O$ $[M + H]^+$: 362.1055, found: 362.1046.

1-(3-Nitrophenyl)-3-(5,6-dihydro-4*H*-1,3-oxazin-2-yl)-9*H*-β-carboline (**9f**)

Yield: 51%; mp 255-258 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 1.96 (qt, *J* 5.5 Hz, 2H, H-5"), 3.59 (t, *J* 5.5 Hz, 2H, H-4"), 4.42 (t, *J* 5.5 Hz, 2H, H-6"), 7.31 (t, *J* 7.2 Hz, 1H, H-6), 7.57-7.67 (m, 2H, H-7, H-8), 7.91 (t, *J* 7.6 Hz, 1H, H-5'), 8.37-8.40 (m, 2H, H-4', H-5), 8.45 (d, *J* 7.6 Hz, 1H, H-6'), 8.76-8.77 (m, 2H, H-2', H-4), 11.86 (s, 1H, NH, H-9); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 21.6 (CH_2, C-5"), 42.1 (CH_2, C-4"), 64.9 (CH_2, C-6"), 112.5 (CH, C-8), 114.0 (CH, C-4), 120.3 (CH, C-6), 121.2 (C_0, C-4b), 122.1 (CH, C-5), 123.2 (CH, C-2'), 123.3 (CH, C-4'), 128.8 (CH, C-7), 130.2 (C_0, C-4a), 130.4 (CH, C-5'), 133.8 (C_0, C-9a), 135.0 (CH, C-6'), 138.3 (C_0, C-1'), 139.5 (C_0, C-8a), 141.4 (C_0, C-3), 141.7 (C_0, C-1), 148.2 (C_0, C-3'), 154.6 (C_0, C-2"); HRMS-ESI *m/z*, calcd. for $C_{21}H_{17}N_4O_3$ $[M + H]^+$: 373.1295, found: 373.1295.

1-(4-Nitrophenyl)-3-(5,6-dihydro-4*H*-1,3-oxazin-2-yl)-9*H*-β-carboline (**9g**)

Yield: 51%; mp 297-300 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 1.96 (qt, *J* 5.5 Hz, 2H, H-5"), 3.59 (t, *J* 5.5 Hz, 2H, H-4"), 4.41 (t, *J* 5.5 Hz, 2H, H-6"), 7.31 (t, *J* 7.8 Hz, 1H, H-6), 7.58-7.68 (m, 2H, H-7, H-8), 8.30 (d, *J* 9.0 Hz, 2H, H-3', H-5'), 8.39 (d, *J* 7.8 Hz, 1H, H-5), 8.46 (d, *J* 9.0 Hz, 2H, H-2', H-6'), 8.79 (s, 1H, H-4), 11.87 (s, 1H, NH, H-9); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 21.6 (CH_2, C-5"), 42.1 (CH_2, C-4"), 64.9 (CH_2, C-6"), 112.5 (CH, C-8), 114.3 (CH, C-4), 120.3 (CH, C-6), 121.1 (C_0, C-4b), 122.0 (CH, C-5), 123.9 (2CH, C-2', C-6'), 128.9 (CH, C-7), 129.8 (2CH, C-3', C-5'), 130.3 (C_0, C-4a), 133.9 (C_0, C-9a), 138.1 (C_0, C-1'), 141.4 (C_0, C-8a), 141.7 (C_0, C-3), 144.3 (C_0, C-1), 147.3 (C_0, C-4'); HRMS-ESI *m/z*, calcd. for $C_{21}H_{17}N_4O_3$ $[M + H]^+$: 373.1295, found: 373.1281.

1-(4-Methoxyphenyl)-3-(5,6-dihydro-4*H*-1,3-oxazin-2-yl)-9*H*-β-carboline (**9h**)

Yield: 66%; mp 219-221 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 2.05 (qt, *J* 5.6 Hz, 2H, H-5"), 3.71 (t, *J* 5.6 Hz, 2H, H-4"), 3.83 (s, 3H, O$\underline{C}H_3$), 4.48 (t, *J* 5.6 Hz, 2H, H-6"), 6.96 (d, *J* 8.2 Hz, 2H, H-3', H-5'), 7.30 (t, *J* 7.8 Hz, 1H, H-6), 7.49-7.54 (m, 2H, H-7, H-8), 7.85 (d, *J* 8.2 Hz, 2H, H-2', H-6'), 8.15 (d, *J* 7.8 Hz, 1H, H-5), 8.63 (s, 1H, H-4), 8.89 (s, 1H, NH, H-9); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 22.0 (CH_2, C-5"), 42.9 (CH_2, C-4"), 55.4 (O$\underline{C}H_3$), 65.6 (CH_2, C-6"), 111.7 (CH, C-8), 112.9 (CH, C-4), 114.3 (2CH, C-3', C-5'), 120.5 (CH, C-6), 121.9 (CH, C-5), 122.3 (C_0, C-4b), 128.4 (CH, C-7), 129.7 (2CH, C-2', C-6'), 129.9 (C_0, C-4a), 130.9 (C_0, C-9a), 134.1 (C_0, C-3), 140.7 (C_0, C-1'), 141.9 (C_0, C-8a), 142.1 (C_0, C-1), 156.0 (C_0, C-4'),

160.1 (C_0, C-2"); HRMS-ESI *m/z*, calcd. for $C_{22}H_{20}N_3O_2$ $[M + H]^+$: 358.1550, found: 358.1567.

Antileishmanial activity

Parasites and cell culture

Leishmania amazonensis promastigotes were maintained at 25 °C in Warren medium supplemented with 10% FBS (fetal bovine serum). J774-A1 macrophages were maintained at 37 °C under 5% CO_2 atmosphere in Roswell Park Memorial Institute (RPMI) 1640 medium (pH 7.2) supplemented with 10% FBS.

Antiprotozoal activity

The effects of synthesized compounds were evaluated in promastigotes of *L. amazonensis* in log phase of growth (48 h) at concentration of 1×10^{-6} cells mL^{-1}. The promastigotes were added into sterile 96-well microplates containing increasing concentrations of compounds **6a**-**6i** and **7a**-**7i**, **8a**-**8i** and **9a**-**9h**. After incubation for 72 h at 25 °C, it was added 50 μL of solution of 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide/phenazine methosulfate (XTT/PMS) (XTT/PMS, 0.5 and 0.3 mg mL^{-1}) in the absence of light. After 4 h, the absorbance was read in a spectrophotometer at 450 nm. The IC_{50} values (concentration that inhibit 50% of cells) was determined by linear regression. Miltefosine was used as positive control.

The effects of compounds **6d**, **8d**, **8i**, **9a**, **9e** and **9h** were also evaluated in intracellular amastigotes, in this antiproliferative assay, J774A1 macrophages (5×10^5 cells mL^{-1}) and promastigotes (5×10^6 parasites mL^{-1}) were added in a plate with coverslips and incubated at 34 °C with 5% CO_2 during 24 h. The treatment was performed after 24 h with compounds in increasing concentrations and incubated for 48 h. For the determination of IC_{50}, the glass coverslips were fixed and stained with Panótico kit as indicated by the manufacturer and 200 macrophages *per* coverslip were evaluated on a light microscope. The number of macrophages infected, the number of amastigotes within each infected macrophage and the survival index (infected cells percentage × amastigote average *per* infected macrophage) were determined. Survival index of amastigotes from untreated infected macrophages was considered as 100% of survival.

Cytotoxicity assay

The cytotoxicity was evaluated in J774-A1 macrophages. The macrophages at concentration of 5×10^{-5} cells mL^{-1} in RPMI 1640 medium supplemented with 10% FBS were introduced into sterile 96-well micro plates and incubated for 24 h at 37 °C and 5% of CO_2 tension. After this period, the supernatant was removed and increasing concentrations of the substances were added. After 48 h of incubation under the same conditions mentioned above, the cells were washed with 0.01 M PBS (phosphate-buffered saline) and 50 μL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) (2 mg mL^{-1}) was added to each well and incubated at absence of light at 34 °C. After 4 h, 150 μL of DMSO was added in order to solubilize formazan crystals. The absorbance was read at 570 nm in microplate reader (Biotek Power Wave XS spectrofluorometer). The concentration that decreased 50% (CC_{50}) of viability of macrophages was determined by linear regression analysis of the data.

Statistical analysis

The data shown in the tables are expressed as the mean ± standard deviation of at least three independent experiments. The statistical analysis was performed using GraphPad Prism 6.0 software.[40] The samples were analyzed using one-way analysis of variance (ANOVA), and the Tukey *post hoc* test was used to compare means when appropriate. Values of $p \leq 0.05$ were considered statistically significant.

Spin labeling and EPR spectroscopy

Promastigotes of *L. amazonensis* in suspension at 5×10^7 parasites mL^{-1} (2 mL) were incubated for 2 h at 26 °C in culture medium without fetal calf serum (FCS) and containing 150 μM of the treatment compound. After incubation, the sample was centrifuged at 1800 × g for 10 min to increase the cell concentration to 1×10^8 parasites mL^{-1} and decrease the final volume to 50 μL. To incorporate the spin label 5-DSA into the parasite membrane first a spin label film was made on the bottom of a glass tube. An aliquot (1 μL) of a 5-DSA ethanolic solution (4 mg mL^{-1}) was added to the tube and after evaporation of ethanol the parasite suspension was placed on the film and stirred gently. Then, the sample was transferred to a 1-mm-i.d. (internal diameter) capillary tube for the EPR measurements.

Spectra were recorded using an EPR EMX-Plus spectrometer of Bruker (Rheinstetten, Germany) with the following spectrometer settings: modulation frequency, 100 kHz; modulation amplitude, 1.0 G; microwave power, 2 mW; magnetic field scan, 100 G; and sample temperature, 25 °C.

Supplementary Information

Supplementary information (HRMS, ^{1}H and ^{13}C NMR spectral data) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

Paula Baréa was responsible for the conceptualization, methodology, data curation, investigation, validation and writing original draft; Jéssica C. de Paula for the methodology, data curation, validation and investigation; Laís Alonso for the methodology, data curation, validation and writing original draft; Aline R. de Oliveira for the methodology and investigation; Willian F. da Costa for the data curation and validation; Antonio Alonso for the formal analysis funding acquisition and resources; Celso V. Nakamura for the investigation, supervision and methodology; Maria H. Sarragiotto for the project administration, conceptualization, formal analysis, funding acquisition, resources, supervision and writing: review and editing.

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