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Article

Synthesis, Antitumor and Antimicrobial Activity of Novel 1-Substituted Phenyl-3-[3-alkylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-Carboline Derivatives

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Com o propósito de aumentar a atividade anticâncer demonstrada anteriormente pelas 1-fenilssubstituído-3-(2-tioxo-1,3,4-oxadiazol-5-il) β-carbolinas **1a**-**c**, neste trabalho foram realizadas a síntese e a avaliação *in vitro* da atividade antitumoral de novas bases de Mannich **2**-**7**(**a**-**c**), derivadas da introdução de diferentes grupos alquilamino(metil) na unidade 1,3,4-oxadiazol de **1a**-**c**. Os derivados **1a**-**c** e **2**-**7**(**a**-**c**) foram também avaliados quanto às atividades antibacteriana e antifúngica. Adicionalmente, um estudo *in silico* das propriedades de ADME dos novos compostos sintetizados **2**-**7**(**a**-**c**) foi realizado pela avaliação de seus parâmetros de Lipinski e de dados de área de superfície topológica polar (TPSA) e de porcentagem de absorção (% ABS).

With the purpose of activity enhancement of 1-substituted phenyl-3-(2-thioxo-1,3,4-oxadiazol-5-yl) β-carbolines **1a**-**c**, reported as potential antitumor agents in our previous study, herein we report the synthesis and antitumor activity evaluation of several novel Mannich bases **2**-**7**(**a**-**c**), by the introduction of different alkylamino(methyl) groups in the 1,3,4-oxadiazole unity of **1a**-**c**. The antimicrobial activities of **1a**-**c** and of **2**-**7**(**a**-**c**) were also evaluated. Additionally, an *in silico* study of the ADME properties of novel synthesized β-carboline derivatives **2**-**7**(**a**-**c**) was performed by evaluation of their Lipinski's parameters and topological polar surface area (TPSA) and percentage of absorption (% ABS) data.

Keywords: 1,3-disubstituted β-carboline derivatives, 1,3,4-oxadiazole, Mannich bases, antitumor activity, antimicrobial activity

Introduction

β-Carboline alkaloids are a class of synthetic and naturally occurring compounds that possess a large spectrum of important pharmacological properties.[1] Particularly, these compounds have shown antitumor activity,[1-10] acting mainly as intercalating into DNA.[6-10] Also, synthetic and natural compounds of this class exhibited antimicrobial activities, mainly against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* bacteria and against *Candida albicans* fungi.[11-13]

Studies on a variety of synthetic β-carboline derivatives have demonstrated the influence of both molecule planarity and substituents nature in C-1, C-3 and position -9 of the β-carboline skeleton on activity.[4,5] The presence of appropriate substituents on these positions could lead to more potent compounds with reduced toxicity.

The biological potential of β-carboline alkaloids and the importance of the search for new antitumor and antimicrobial agents have led us to study this class of compounds. In previous work, we reported synthesis and *in vitro* antitumor activities of a series of 1-substituted phenyl β-carboline bearing 2-thioxo-1,3,4-oxadiazol-5-yl moiety at C-3.[14] The anticancer assay results pointed compounds **1a**-**c** with growth inhibition effect (GI_{50} < 100 μmol L^{-1}) for all eight human cancer cell lines tested.[14]

Taking in account our previous results and with the aim of increasing the antitumor activity we have continued our

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work on β-carboline derivatives **1**, with the introduction of substituents in 1,3,4-oxadiazole ring. Literature reports that Mannich bases of oxadiazoles possess important activities, such as antibacterial and anticancer.[15-17]

Therefore, in this study we synthesized six series of Mannich bases from the previously reported active compounds **1a-c**[14] to investigate the effects of different 3-alkylamino(methyl) substituents on the oxadiazole ring, expecting that the incorporation of these substituents would lead to antitumor activity enhancement. More specifically, we report the synthesis and *in vitro* anticancer activity of β-carboline derivatives **2-7**(**a-c**) bearing a 3-alkylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl group at C-3. Additionally, the antimicrobial activities of **1a-c** and of the Mannich bases **2-7**(**a-c**) were evaluated.

An *in silico* study of the ADME properties of novel synthesized β-carboline derivatives was carried out by investigation of their Lipinski's parameters, topological polar surface area (TPSA) and percentage of absorption (% ABS).[18,19]

Results and Discussion

Chemistry

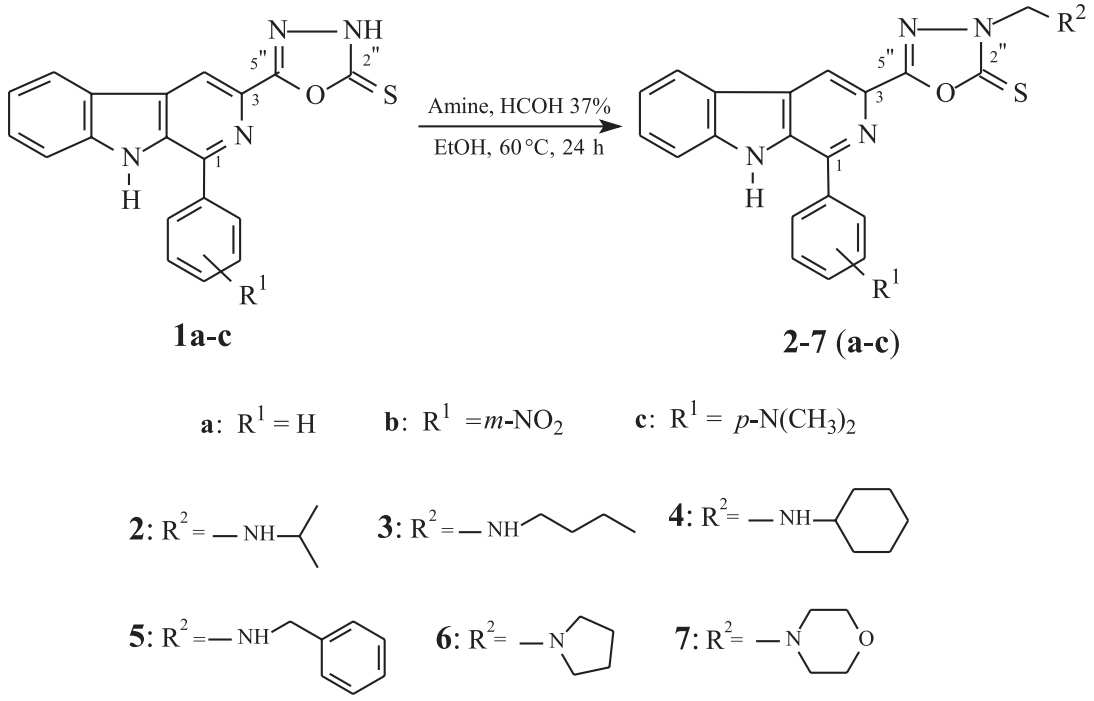
The 3-(2-thioxo-1,3,4-oxadiazol-5-yl) β-carbolines **1a-c** were prepared from the commercial *L*-tryptophan as previously reported.[14] The condensation reaction of **1a-c**, formaldehyde 37%[15,17] and the primary amines isopropylamine, butylamine, cyclohexylamine and benzylamine, using ethanol as solvent, afforded the Mannich bases **2a-c**, **3a-c**, **4a-c** and **5a-c**, respectively (Scheme 1). Condensation reaction with pyrrolidine and morpholine secondary amines under the same conditions, afforded the Mannich bases **6a-c** and **7a-c**, respectively (Scheme 1). The Mannich bases **2-7**(**a-c**) were obtained in range of 52% to 92% yields from **1a-c**, and in six steps from commercial *L*-tryptophan with 15-34% overall yields.

All novel compounds were characterized by their spectroscopic data (IR, EIMS, ^{1}H and ^{13}C NMR), which are described in the Experimental section.

The formation of Mannich bases **2-7**(**a-c**) was evidenced by the presence of a singlet at δ_H 5.00-6.00 in the ^{1}H NMR spectra, corresponding to the methylene exocyclic hydrogens of the alkylamino(methyl) group introduced at *N*-3 of the 1,3,4-oxadiazole ring, together with the signals for each group alkylamino. The exocyclic methylene carbon of the alkylamino group appears in the region of δ_C 60-70 in the ^{13}C NMR. The signals in the δ_C 177 and 160 region were assigned for C-2 and C-5 carbons, respectively, of the 1,3,4-oxadiazole ring.

The IR spectra showed absorption bands characteristic for N-H indole and C=N stretching in the 3040 and 1615 cm^{-1} region, respectively, and for C=S group at 1390 and 1240 cm^{-1}.

The structures of **2-7**(**a-c**) were also confirmed by EI mass spectra. The majority of compounds showed the presence of a base peak at *m/z* [M$^{+\cdot}$- alkylamino(methyl)-



Scheme 1. Synthesis of compounds **2-7** (**a-c**).

thioxo-1,3,4-oxadiazole] relative to cleavage between C-3 of β-carboline and C-5 of 1,3,4-oxadiazole ring. For the benzyl derivatives **5a**-**c** the base peaks were observed at *m/z* 91, which was due to the tropilium ion formation.

Anticancer activity

The GI_{50}, TGI and LC_{50} data and MG-MID values obtained for each compound and cell lines tested were summarized in Tables 1 and 2. The anticancer assay results pointed towards eleven compounds (**2a**, **2c**, **3a**, **5b**, **5c**, **6a**-**c** and **7a**-**c**) with growth inhibition effect ($GI_{50} < 100$ μmol L^{-1}) for all eight human cancer cell lines tested, showing GI_{50} (MG-MID) values within 4.37 to 36.31 μmol L^{-1} range (Table 1). Analysis of the TGI (MG-MID) values (Table 2) showed that the Mannich bases synthesized were able to inhibit total growth (cytostatic activity), except for **3b** and **7c**, in a concentration minor than 100 μmol L^{-1}.

Among compounds tested, **2a**, **2c** and **5c** were the most active derivatives, exhibiting a broad antitumor activity spectrum at GI_{50} and TGI levels, with GI_{50} (MG-MID) values of 5.89, 4.37 and 4.57 μmol L^{-1} (Table 1), respectively. Moreover, compound **2a** displayed cytotoxic efficacy with LC_{50} (MG-MID) value of 60.49 μmol L^{-1} (Table 2).

A potent activity against melanoma (UACC-62) and lung (NCI-460) cell lines, with GI_{50} values of 0.88 and 1.01 μmol L^{-1} (Table 1), respectively, was observed for compound **2c**, which possess the N,N-dimethylphenyl and isopropylamino(methyl) groups at C-1 and C-3, respectively, of the β-carboline nucleus. Compound **5c** also demonstrated a significant activity, with GI_{50} values in the range of 0.38 and 2.98 μmol L^{-1} towards four cell lines (melanoma, ovarian resistant, renal and lung).

Comparison of the GI_{50} (MG-MID) values for all tested compounds showed that, except for **3b**, **4c** and **7c,** the Mannich bases derivatives displayed higher anticancer activity than the corresponding precursors. For the most active **2a**, **2c** and **5c** Mannich bases**,** the introduction of an alkylamino(methyl) group at N-3 of the heterocyclic ring

Table 1. GI_{50} values (in μmol L^{-1}) and GI_{50} MG-MID (in μmol L^{-1}) for compounds **1a**-**c** and **2**-**7**(**a**-**c**)

Compounds	Cancer cell line								
	Melanoma UACC-62	Breast MCF7	Ovarian resistant NCI/ADR	Renal 786-0	Lung NCI-460	Prostate PCO-3	Ovarian OVCAR	Colon HT29	MG-MID[a]
Doxorubicin	0.43	0.005	0.13	0.21	0.04	0.46	0.34	0.46	0.14
1a	>100	25.54	17.60	27.95	20.60	25.54	31.77	25.54	29.25
1b	24.10	27.93	74.99	32.38	73.41	12.20	22.31	27.93	31.26
1c	23.82	18.84	32.11	14.72	19.09	14.72	14.72	40.46	20.83
2a	6.00	0.86	5.90	4.66	5.72	6.12	6.21	43.85	5.89
2b	7.08	4.56	9.27	49.90	4.26	53.54	6.25	>100	14.79
2c	0.88	5.88	2.78	9.82	1.01	13.31	3.42	22.06	4.37
3a	5.29	7.29	30.16	6.51	2.74	26.66	5.99	49.80	10.72
3b	>100	>100	>100	>100	>100	>100	>100	>100	100.00
3c	10.34	42.04	16.32	5.03	3.99	35.24	39.05	>100	19.50
4a	8.89	40.79	13.49	28.57	7.02	38.99	11.21	>100	21.38
4b	7.58	14.33	3.74	49.81	4.33	20.31	12.28	>100	14.79
4c	8.74	>100	5.13	52.44	15.19	58.48	18.66	>100	28.18
5a	>100	16.87	3.72	1.31	4.63	47.68	6.39	54.29	12.59
5b	11.30	16.64	5.34	7.13	5.25	14.17	5.55	68.52	10.96
5c	2.98	10.23	2.55	0.38	1.28	14.96	5.91	56.34	4.57
6a	7.61	22.81	6.83	9.45	7.58	22.64	6.97	21.53	11.48
6b	6.97	11.23	6.65	15.98	17.98	14.25	6.51	24.15	11.75
6c	6.63	16.08	7.05	21.81	11.04	11.00	5.96	14.13	10.72
7a	6.77	39.43	7.59	11.42	36.77	8.10	7.38	17.61	13.18
7b	6.81	11.49	7.96	51.73	40.43	6.33	10.38	38.91	15.49
7c	24.19	56.35	20.16	67.63	38.55	16.58	47.33	49.28	36.31

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

Table 2. TGI (in μmol L^{-1}) and LC_{50} (in μmol L^{-1}, values in parentheses) and TGI and LC_{50} MG-MID (μmol L^{-1}) for compounds **1a-c** and **2-7**(**a-c**)

Compounds	Cancer cell line								
	Melanoma UACC-62	Breast MCF7	Ovarian resistant NCI/ADR	Renal 786-0	Lung NCI 460	Prostate PCO-3	Ovarian OVCAR	Colon HT29	MG-MID[a]
Doxorubicin	0.44 (0.99)	0.09 (2.78)	6.14 (68.44)	10.00 (>100)	1.25 (>100)	4.59 (82.12)	2.61 (>100)	10.58 (>100)	2.09 (33.37)
1a	>100	>100	>100	>100	>100	>100	>100	>100	>100 (>100)
1b	>100	>100	>100	>100	>100	35.77(>100)	>100	>100	87.1 (>100)
1c	>100	>100	>100	>100	>100	>100	>100	>100	>100 (>100)
2a	15.63 (56.88)	16.01 (74.61)	20.10 (63.05)	3.96 (20.62)	17.63 (61.95)	30.91 (>100)	14.64 (52.41)	81.39 (>100)	18.36 (60.49)
2b	>100	98.35 (>100)	>100	>100	88.90 (>100)	>100	67.50 (>100)	>100	93.62 (>100)
2c	6.81 (>100)	>100	39.16	>100	>100	>100	95.97 (>100)	>100	63.25 (>100)
3a	15.12 (58.99)	>100	>100	43.71 (>100)	>100	>100	25.59 (>100)	>100	60.05 (93.62)
3b	>100	>100	>100	>100	>100	>100	>100	>100	>100 (>100)
3c	57.49 (>100)	>100	>100	21.55 (>100)	>100	>100	>100	>100	77.03 (>100)
4a	32.14 (52.07)	>100	>100	>100	>100	>100	>100	>100	86.77 (92.17)
4b	65.59 (48.62)	>100	>100	>100	>100	>100	>100	>100	94.86 (92.17)
4c	35.12 (>100)	>100	>100	>100	>100	>100	>100	>100	87.74 (>100)
5a	91.16 (>100)	66.96 (>100)	37.97 (>100)	12.94 (52.62)	39.85 (>100)	72.49 (>100)	34.54 (>100)	>100	48.36 (92.29)
5b	53.52 (>100)	68.48 (>100)	52.52 (>100)	21.66 (41.14)	>100	56.83 (>100)	22.25 (>100)	>100	51.91 (89.49)
5c	58.52 (>100)	>100	94.87 (>100)	19.62 (>100)	>100	>100	58.91 (>100)	>100	70.94 (>100)
6a	34.17 (>100)	>100	51.40 (>100)	>100	>100	72.79 (>100)	27.24 (57.73)	75.33 (>100)	63.44 (93.36)
6b	21.31 (47.18)	>100	46.48 (>100)	>100	>100	>100	21.53 (49.91)	91.41 (>100)	61.13 (83.18)
6c	25.34 (52.70)	>100	91.37 (>100)	77.82 (>100)	71.19 (>100)	60.35 (>100)	24.81 (>100)	65.45 (>100)	57.86 (93.33)
7a	26.24 (53.19)	>100	>100	37.81 (>100)	>100	63.76 (>100)	28.81 (56.37)	71.36 (>100)	58.11 (86.02)
7b	>100	>100	>100	>100	46.84 (>100)	30.11(>100)	44.97(69.85)	57.36 (>100)	66.09 (95.61)
7c	>100	>100	>100	>100	>100	>100	>100	>100	>100 (>100)

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

improved antitumoral activity 4.5 timefold compared to those of the 1,3,4-oxadiazoles **1a** and **1c**. Also, concerning to the effect of the different alkylamino groups on activity we observed that the isopropylamino(methyl) and benzylamino(methyl) substituents were the best choice for an increase of activity.

Antimicrobial activity

Compounds **1a-c** and their Mannich bases **2-7**(**a-c**) were assayed against the bacteria *Bacillus subtilis* ATCC 2576, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 13388, *Sthaphylococus aureus* ATCC 6538; and against the fungi *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC-22019 and *Candida tropicalis* ATCC-28707.

The bioassays results for compounds **1a-c** showed a potent antifungal activity for compound **1b** against the *Candida albicans* fungi, with MIC value of 3.1 μg mL^{-1} This derivative and the compound **1a** were weakly active towards the bacteria *B. subtilis* (MIC = 250 μg mL^{-1}). For the Mannich bases **2-7**(**a-c**) none of the derivatives presented activity at the maximum concentration tested (250 μg mL^{-1}), which demonstrated that the introduction of alkylamino(methyl) groups at the oxadiazole ring of **1a-c** did not contribute for antimicrobial activity.

In silico study

An *in silico* computational study of the synthesized 1-phenyl substituted-3-(3-alkylaminomethyl-2-thioxo-1,3,4-oxadiazol-5-yl) β-carbolines **2-7**(**a-c**) was performed by determination of Lipinski's parameters, topological polar surface area (TPSA) and percentage of absorption (% ABS). Calculations were performed using "Molinspiration online property calculation toolkit" (http://www.molinspiration.com)[20] and "OSIRIS property explorer" (www.organic-chemistry.org/prog/peo).[21] The percentage of absorption was estimated using equation: %ABS = 109 − 0.345 × TPSA, according to Zhao *et al.*[19] These data are shown in Table 3.

Table 3. Lipinsk´s parameters and %ABS, TPSA, Log S for compounds **2-7** (**a-c**)

Comp	% ABS	TPSA (Å²)	Lipinski's parameters					Log S
			nALH acceptors	nDLH donors	milogP	MW	n violations	
2a	84.27	71.68	6	2	4.31	415.52	0	-6.51
2b	68.46	117.50	9	2	4.27	460.52	0	-6.97
2c	83.15	74.91	7	2	4.42	458.59	0	-6.55
3a	84.27	71.676	6	2	5.08	429.55	1	-6.67
3b	68.46	117.5	9	2	5.04	474.55	1	-7.13
3c	83.15	74.914	7	2	5.18	472.62	1	-6.71
4a	84.27	71.68	6	2	5.55	455.59	1	-7.41
4b	68.46	117.50	9	2	5.50	500.58	2	-6.38
4c	83.15	74.91	7	2	5.65	498.66	1	-7.44
5a	84.27	71.68	6	2	5.04	463.57	1	-7.16
5b	68.46	117.50	9	2	5.00	508.56	1	-6.38
5c	83.15	74.91	7	2	5.14	506.64	2	-7.19
6a	87.3	62.89	6	1	4.29	427.53	0	-6.16
6b	71.49	108.71	9	1	4.25	472.53	0	-6.62
6c	86.19	66.13	7	1	4.39	470.60	0	-6.19
7a	84.12	72.12	7	1	3.73	443.53	0	-5.54
7b	68.31	117.95	10	1	3.69	488.53	0	-6.00
7c	83.00	75.36	8	1	3.83	486.60	0	-5.57

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: (*i*) hydrogen bond donors ≤ 5 (OH and NH groups); (*ii*) hydrogen bond acceptors ≤ 10 (N and O atoms); (*iii*) molecular weight < 500; (*iv*) calculated log P < 5.[18-20] Compounds violating more than one of these rules may present bioavailability problems.

Our results (Table 3) revealed that the Mannich base derivatives containing the isopropylamino(methyl) (**2a-c**), pyrrolidyl(methyl) (**6a-c**) and morpholyl(methyl) (7**a-c**) groups attached to the 1,3,4-oxadiazole ring presented lipophilicity minor than 5, with values between 3.69 and 4.41. On the other hand, the derivatives containing the butylamino(methyl) (**3a-c**), cyclohexylamino(methyl) (**4a-c**) and benzylamino(methyl) (**5a-c**) groups showed lipophilicity larger than 5.0, violating one of the Lipinski's rules.

Except for **4b**, **5b** e **5c** the molecular weight were minor than 500 (415.52 > MW < 498.65). All Mannich base derivatives have number of hydrogen bond acceptors (*n*-ON = 6-10) and donors (*n*-OHNH = 1-2) in agreement with Lipinski's rule. The calculated percent absorption (% ABS) of all derivatives ranged between 68.31 and 87.30 %, indicating that these compounds have a good permeability in the cellular plasmatic membrane. In summary, *in silico* study pointed the Mannich bases synthesized in our work as potential candidates for new antitumor agents.

Conclusions

In conclusion, several 1-substituted phenyl-β-carbolines containing a 3-alkylamino(methyl)-2-thioxo-1,3,4-oxadiazolyl group were prepared and identified as novel antitumor agents. Compounds **2a**, **2c** and **5c** were the most active derivatives, exhibiting a broad spectrum antitumor activity at GI_{50} and TGI levels. Except for **3b**, **4c** and **7c**, the Mannich base derivatives displayed higher anticancer activity than their corresponding precursors. For the most active Mannich bases **2a**, **2c** and **5c**, the introduction of an alkylamino(methyl) group at *N*-3 of the heterocyclic ring improved antitumor activity 4.5 timefold compared to those of the 1,3,4-oxadiazoles **1a** and **1c**.

The antimicrobial bioassays results showed that, among all tested compounds, only compound **1b** was active, displaying potent activity against the yeast *C. albicans*.

Finally, *in silico* study pointed the novel derivatives as potential candidates for new drugs.

Experimental

General

^{1}H and ^{13}C spectra were recorded in a Varian spectrometer model Mercury plus BB at 300 MHz and 75.5 MHz, respectively, with DMSO-d_6 as solvent and TMS as the internal standard. Mass spectra (MS) were recorded in a Thermoelectron Corporation Focus-DSQ II spectrometer. IR spectra were recorded on a BOMEM spectrometer model MB-100. For TLC, Merck precoated plates (silica gel 60 G254) were used. Silica gel 60 Merck (230-400 mesh) was used in column chromatography purification of some compounds. All reagents were purchased from commercial suppliers.

General procedure for preparation of 1-(substituted phenyl)-3-[2-thioxo-3-alkylamino(methyl)-1,3,4-oxadiazol-5-yl] β-*carbolines* ***2-7(a-c)***

The 1-(substituted phenyl)-3-(2-thioxo-1,3,4-oxadiazol-5-yl) β-carbolines **1a-c** were prepared as previously reported.[14]

To a solution of derivatives **1a-c** (0.5 mmol) in ethanol (10 mL) the primary or secondary amines (0.5 mmol) were added, followed of the dropwise addition of formaldehyde (37%, 3.0 mmol). The solution was stirred for 24 h at 60 °C and then, for 1h at 0 °C. The precipitated solid was filtered under vacuum suction and washed with cold ethanol. The characterization data of the Mannich bases obtained are given bellow.

1-Phenyl-3-[3-isopropylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-*carboline (**2a**)*

Yield: 62%, mp 234.0-237.0 °C. IR (KBr) ν_{max} /cm^{-1}: 3040 (N–H), 1614 (C=N), 1360 and 1239 (C=S), 1565, 1487 and 1464 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.94 (s, 1H, H-4), 8.47 (d, *J* 7.8 Hz, 1H, H-5), 7.35 (t, *J* 7.8 Hz, 1H, H-6), 7.57-7.73 (m, 5H, H-7, H-8, H-3', H-4' and H-5'), 12.00 (s, 1H, NH-9), 8.09 (d, *J* 6.9 Hz, 2H, H-2' and H-6'), 5.99 (brs, 2H, CH_2-exocyclic), 11.43 (brs, 1H, NH-isopropyl), 4.60 (hep, *J* 6.9 Hz, 1H, CH-isopropyl), 1.22 (d, *J* 6.9 Hz, 6H, CH_3-isopropyl). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 141.06 (C-1), 138.9 (C-3), 115.9 (C-4), 128.8 (C-4a), 121.1 (C-4b), 122.2 (C-5), 120.5 (C-6), 129.2 (C-7), 112.8 (C-8), 140.4 (C-8a), 133.9 (C-9a), 129.9 (C-1'), 128.5 (C-2'/6'), 129.0 (C-3'/5'), 128.9 (C-4'), 174.3 (C-2"), 159.7 (C-5"), 64.6 (CH_2-exocyclic), 46.2 (CH-isopropyl), 18.9 (CH_3-isopropyl). EIMS, 70 eV, *m/z* (rel. int., %): 415 (10, $M^{+\cdot}$), 344 (10), 271 (20), 243 (100), 121 (5), 100 (5).

1-(3-Nitrophenyl)-3-[3-isopropylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-*carboline (**2b**)*

Yield: 89%, mp 236.0- 237.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3054 (N–H), 1611 (C=N), 1350 and 1239 (C=S), 1566, 1485 and 1465 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.01 (s, 1H, H-4), 8.55 (d, *J* 7.8 Hz, 1H, H-5), 7.37 (t, *J* 7.8 Hz, 1H, H-6), 7.63-7.73 (m, 2H, H-7, H-8), 12.17 (s, 1H, 9-NH), 8.83 (s, 1H, H-2'), 8.51 (d, *J* 7.8 Hz, H-4'), 7.97 (t, *J* 7.8 Hz, H-5'), 8.44 (dd, *J* 7.8 Hz, 1.8 Hz, H-6'), 6.00 (brs, 2H, CH_2-exocyclic), 11.46 (brs, 1H, NH-isopropyl), 4.63 (hep, *J* 6.9 Hz, 1H, CH-isopropyl), 1.23 (d, *J* 6.9 Hz, 6H, CH_3-isopropyl). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 141.7 (C-1), 137.9 (C-3), 116.7 (C-4), 130.5 (C-4a), 121.0 (C-4b), 122.4 (C-5), 120.7 (C-6), 129.2 (C-7), 112.7 (C-8), 139.09 (C-8a), 139.0 (C-9a), 134.1 (C-1'), 123.3 (C-2'), 148.3 (C-3'), 123.6 (C-4'), 130.5 (C-5'), 134.9 (C-6'), 174.3 (C-2"), 159.9 (C-5"), 64.6 (CH_2-exocyclic), 46.2 (CH-isopropyl), 18.9 (CH_3-isopropyl). EIMS, 70 eV, *m/z* (rel. int., %): 460 (5, $M^{+\cdot}$), 344 (15), 289 (95), 242 (100), 121(30), 100 (75).

1-(4-N,N-Dimethylaminophenyl)-3-[3-isopropylamino (methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-*carboline (**2c**)*

Yield: 90%, mp 160.0-161.0 °C. IR (KBr) ν_{max}/ cm^{-1}: 3085 (N–H), 1609 (C=N), 1365 and 1240 (C=S), 1557, 1488 and 1466 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.82 (s, 1H, H-4), 8.42 (d, *J* 7.8 Hz, 1H, H-5), 7.32 (t, *J* 7.8 Hz, H-6), 7.60 (t, *J* 7.8 Hz, H-7), 7.72 (d, *J* 7.8 Hz, H-8), 11.87 (s, 1H, 9-NH), 7.95 (d, *J* 8.7 Hz, 2H, H-2' and H-6'), 6.96 (d, *J* 8.7 Hz, 2H, H-3' and H-5'), 3.05 (s, 6H, $N(CH_3)_2$), 5.98 (brs, 2H, CH_2- exocyclic), 11.40 (brs, 1H, NH-isopropyl), 4.61 (hep, *J* 6.6 Hz, 1H, CH-isopropyl), 1.24 (d, *J* 6.6 Hz, 6H, CH_3-isopropyl). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 141.5 (C-1), 138.7 (C-3), 114.7 (C-4), 124.9 (C-4a), 121.2 (C-4b), 122.0 (C-5), 120.3 (C-6), 128.6 (C-7), 112.8 (C-8), 141.3 (C-8a), 133.5 (C-9a), 129.4 (C-1'), 129.3 (C-2'), 112.2 (C-3'), 150.8 (C-4'), 112.2 (C-5'), 129.3 (C-6'), 40.0 - $N(CH_3)_2$, 174.3 (C-2"), 166.7 (C-5"), 64.6 (CH_2-exocyclic), 46.2 (CH-isopropyl), 19.0 (CH_3-isopropyl). EIMS, 70 eV, *m/z* (rel. int., %): 458 (5, $M^{+\cdot}$), 344 (10), 287 (100), 242 (45), 121 (25), 100 (45).

*1-Phenyl-3-[3-butylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl]*β-*carboline (**3a**)*

Yield: 83%, mp 212.0-215.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3057 (N–H), 1618 (C=N), 1394 and 1238 (C=S), 1566, 1492 and 1456 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.93 (s, 1H, H-4), 8.46 (d, *J* 7.8 Hz, 1H, H-5), 7.35 (t, *J* 7.8 Hz, 1H, H-6), 7.57-7.73 (m, 5H, H-7, H-8, H-3', H-4' and H-5'), 11.99 (s, 1H, 9-NH), 8.08 (d, *J* 6.9 Hz, 2H, H-2' and H-6'), 5.92 (brs, 2H, CH_2-exocyclic), 11.44 (brs, 1H,

NH-butyl), 3.58 (t, *J* 7.2 Hz, 2H, CH_2-butyl), 1.56 (quint., *J* 7.2 Hz, 2H, CH_2-butyl), 1.31 (sext., *J* 7.2 Hz, 2H, CH_2-butyl), 0.90 (t, *J* 7.2 Hz, 3H, CH_3-butyl). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 141.6 (C-1), 138.9 (C-3), 115.9 (C-4), 129.9 (C-4a), 121.1 (C-4b), 122.2 (C-5), 120.4 (C-6), 129.2 (C-7), 112.8 (C-8), 140.5 (C-8a), 137.5 (C-9a), 133.9 (C-1'), 128.5 (C-2'/6'), 129.0 (C-3'/5'), 128.9 (C-4'), 175.5 (C-2"), 160.5 (C-5"), 68.0 (CH_2-exocyclic), 43.9 (CH_2-butyl), 28.4 (CH_2- butyl), 19.4 (CH_2- butyl), 13.6 (CH_3-butyl). EIMS, 70 eV, *m/z* (rel. int., %): 429 (5, $M^{+\cdot}$), 344 (25), 243 (100), 157 (15), 102 (15).

1-(3-Nitrophenyl)-3-[3-butylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-*carboline (**3b**)*

Yield: 65%, mp 230.0-235.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3053 (N–H), 1612 (C=N), 1345 and 1238 (C=S), 1565 and 1454 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.00 (s, 1H, H-4); 8.55 (d, *J* 7.8 Hz,1H, H-5), 7.37 (t, *J* 7.8 Hz, 1H, H-6), 7.63 - 7.73 (m, 2H, H-7, H-8), 12.17 (s, 1H, 9-NH), 8.83 (s, 1H, H-2'), 8.50 (d, *J* 8.0 Hz, 1H, H-4'), 7.96 (t, *J* 8.0 Hz, 1H, H-5'), 8.43 (dd, *J* 8.0 and 2.1 Hz, 1H, H-6'), 5.98 (brs, 2H, CH_2-exocyclic), 11.47 (brs, 1H, NH-butyl), 3.59 (t, *J* 7.2 Hz, 2H, CH_2-butyl), 1.59 (quint., *J* 7.2 Hz, 2H, CH_2-butyl), 1.31 (sext., *J* 7.2 Hz, 2H, CH_2-butyl), 0.90 (t, *J* 7.2 Hz, 3H, CH_3-butyl). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 141.7 (C-1), 138.9 (C-3), 116.7 (C-4), 121.0 (C_0-4b), 123.6 (C-5), 120.7 (C-6), 129.2 (C-7), 112.7 (C-8), 139.1 (C-8a), 137.8 (C-9a), 134.1 (C-1'), 123.4 (C-2'), 148.3 (C-3'), 122.4 (C-4'), 130.5 (C-5'), 134.8 (C-6'), 175.4 (C-2"), 158.5 (C-5"), 68.5 (CH_2-exocyclic), 44.1 (CH_2-butyl), 28.3 (CH_2-butyl), 19.5 (CH_2-butyl), 13.6 (CH_3-butyl). EIMS, 70 eV, *m/z* (rel. int., %): 474 (5, $M^{+\cdot}$), 242 (15), 157 (95), 102 (75), 57 (100).

1-(4-N,N-Dimethylaminophenyl)-3-[3-butylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-*carboline (**3c**)*

Yield: 92%, mp 214.0-216 °C. IR (KBr) ν_{max}/cm^{-1}: 3262 (N-H); 1608 (C=N); 1348 and 1229 (C=S), 1558, 1493 and 1452 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.80 (s, 1H, H-4), 8.42 (d, *J* 7.8 Hz, 1H, H-5), 7.32 (t, *J* 7.8 Hz, 1H, H-6), 7.60 (t, *J* 7.8 Hz, 1H, H-7), 7.71 (d, *J* 7.8 Hz, 1H, H-8), 11.86 (s, 1H, 9-NH), 7.95 (d, *J* 8.7 Hz, 1H, H-2'), 6.96 (d, *J* 8.7 Hz, 1H, H-3'), 6.96 (d, *J* 8.7 Hz, 1H, H-5'), 7.95 (d, *J* 8.7 Hz, 1H, H-6'), 3.05 (s, 6H, $N(CH_3)_2$), 5.92 (brs, 2H, CH_2-exocyclic), 11.41 (brs, 1H, NH-butyl), 3.59 (t, *J* 7.3 Hz, 2H, CH_2-butyl), 1.57 (quint., *J* 7.3 Hz, 2H, CH_2-butyl), 1.32 (sext., *J* 7.3 Hz, 2H, CH_2-butyl), 0.92 (t, *J* 7.3 Hz, 3H, CH_3-butyl). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 141.4 (C-1), 138.7 (C-3), 114.7 (C-4), 124.9 (C-4a), 121.2 (C-4b), 122.0 (C-5), 120.3 (C-6), 128.6 (C-7), 112.8 (C-8), 141.3 (C-8a), 129.3 (C-9a), 133.5 (C-1'), 129.3 (C-2'), 112.2 (C-3'), 150.8 (C-4'), 112.2 -(C-5'), 129.3 (C-6'), 175.5 (C-2"), 164.6 (C-5"), 40.0 - $(CH_3)_2$, 68.2 (CH_2-exocyclic), 44.0 (CH_2-butyl), 28.5 (CH_2-butyl), 19.5 (CH_2-butyl), 13.7 (CH_3-butyl). EIMS, 70 eV, *m/z* (rel. int., %): 472 (5, $M^{+\cdot}$), 242 (25), 157 (70), 84 (25), 76 (100).

1-Phenyl-3-[3-cyclohexylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-*carboline (**4a**)*

Yield: 74%, mp 222.0-224.0 °C. IR (KBr) ν_{max}/ cm^{-1}: 3042 (N–H), 1613 (C=N), 1372 and 1237 (C=S), 1564, 1484 and 1453 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.94 (s, 1H, H-4), 8.45 (d, *J* 7.8 Hz, 1H, H-5), 7.35 (t, *J* 7.8 Hz, 1H, H-6), 7.58 - 7.73 (m, 5H, H-7, H-8, H-3', H-4' and H-5'), 12.00 (s, 1H, 9-NH), 8.10 (d, *J* 6.9 Hz, 2H, H-2' and H-6'), 6.00 (brs, 2H, CH_2-exocyclic), 11.40 (brs, 1H, NH-cyclohexyl), 4.21 (m, 1H, CH-cyclohexyl), 1.03-1.84 (m, 10H, CH_2-cyclohexyl). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 141.6 (C-1), 138.9 (C-3), 115.8 (C-4), 128.7 (C-4a), 121.1 (C-4b), 122.2 (C-5), 120.4 (C-6), 129.2 (C-7), 112.8 (C-8), 140.3 (C-8a), 133.8 (C-9a), 130.0 (C-1'), 128.5 (C-2'/6'), 128.9 (C-3'/5'), 128.8 (C-4'), 177.6 (C-2"), 166.8 (C-5"), 65.3 (CH_2-exocyclic), 53.7 (CH-cyclohexyl), 29.1 (CH_2-cyclohexyl), 25.0 (CH_2-cyclohexyl). EIMS, 70 eV, *m/z* (rel. int., %): 455 (5, $M^{+\cdot}$), 243 (100), 183 (10), 102 (25), 55 (30).

1-(3-Nitrophenyl)-3-[3-cyclohexylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-*carboline (**4b**)*

Yield: 83%, mp 222.0-223.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3064 (N–H), 1613 (C=N), 1346 and 1239 (C=S), 1559, 1485 and 1459 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.00 (s, 1H, H-4), 8.55 (d, *J* 7.8 Hz, 1H, H-5), 7.37 (t, *J* 7.8 Hz, 1H, H-6), 7.63-7.73 (m, 2H, H-7 and H-8), 12.17 (s, 1H, 9-NH), 8.82 (brs, 1H, H-2'), 8.50 (d, *J* 8.1 Hz, 1H, H-4'), 7.97 (t, *J* 8.1 Hz, 1H, H-5'), 8.44 (d, *J* 8.1 Hz,1H, H-6'), 6.02 (brs, 2H, CH_2-exocyclic), 11.46 (brs, 1H, NH-cyclohexyl-), 4.22 (m, 1H, CH-cyclohexyl), 1.03-1.85 (m, 10H, CH_2-cyclohexyl). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 141.7 (C-1), 139.1 (C-3), 116.7 (C-4), 121.0 (C-4b), 122.4 (C-5), 120.6 (C-6), 129.2 (C-7), 112.7 (C-8), 139.7 (C-8a), 137.9 (C-9a), 134.1 (C-1'), 123.4 (C-2'), 148.4 (C-3'), 123.6 (C-4'), 130.5 (C-5'), 134.9 (C-6'), 174.2 (C-2"), 164.9 (C-5"), 65.4 (CH_2-exocyclic), 53.8 (CH-cyclohexyl), 28.9 (CH_2-cyclohexyl), 24.9 (CH_2-cyclohexyl), 25.1 (CH_2-cyclohexyl), 24.6 (CH_2-cyclohexyl). EIMS, 70 eV, *m/z* (rel. int., %): 500 (5, $M^{+\cdot}$), 242 (60), 183 (30), 102 (100), 55 (90).

1-(4-N,N-Dimethylaminophenyl-3-[3-cyclohexylamino (methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-*carboline (**4c**)*

Yield: 70%, mp 173.0-175.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3083 (N–H), 1609 (C=N), 1361 and 1240 (C=S), 1555, 1489 and 1450 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6):

δ 8.81 (s, 1H, H-4), 8.42 (d, *J* 7.8 Hz, 1H, H-5), 7.32 (t, *J* 7.8 Hz, 1H, H-6), 7.60 (t, *J* 7.8 Hz, 1H, H-7), 7.71 (d, *J* 9.0 Hz, 1H, H-8), 11.88 (s, 1H, 9 -NH), 7.97 (d, *J* 9.0 Hz, 1H, H-2'), 6.96 (d, *J* 9.0 Hz, 1H, H-3'), 6.96 (d, *J* 9.0 Hz, 1H, H-5'), 7.97 (d, *J* 9.0 Hz, 1H, H-6'), 3.05 (s, 6H, $N(CH_3)_2$), 5.99 (brs, 2H, CH_2-exocyclic), 11.41 (brs, 1H, NH-cyclohexyl), 4.21 (m, 1H, CH-cyclohexyl), 1.03-1.84 (m, 10H, CH_2-cyclohexyl). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 141.4 (C-1), 138.7 (C-3), 114.6 (C-4), 124.9 (C-4a), 121.2 (C-4b), 122.0 (C-5), 120.3 (C-6), 128.5 (C-7), 112.7 (C-8), 141.2 (C-8a), 133.4 (C-9a), 129.4 (C-1'), 129.3 (C-2'), 112.1 (C-3'), 150.8 (C-4'), 112.1 (C-5'), 129.3 (C-6'), 174.2 (C-2"), 39.9 $-N(CH_3)_2$, 65.4 (CH_2-exocyclic), 53.7 (CH-cyclohexyl), 29.1 (CH_2-cyclohexyl), 25.0 (CH_2-cyclohexyl). EIMS, 70 eV, *m/z* (rel. int., %): 498 (5, $M^{+\cdot}$), 183 (5), 83 (55), 57 (100).

1-Phenyl-3-[3-benzylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-*carboline (**5a**)*

Yield: 79%, mp 225.0-228.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3062 (N–H), 1618 (C=N), 1396 and 1237 (C=S), 1565, 1493, and 1454 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.91 (s, 1H, H-4), 8.45 (d, *J* 7.8 Hz, 1H, H-5), 7.31-7.38 (m, 1H, H-6), 7.60-7.64 (m, 1H, H-7), 7.70 (d, *J* 7.8 Hz, 1H, H-8), 11.96 (brs, 1H, 9-NH), 7.89 (d, *J* 6.9 Hz, 2H, H-2' and H-6'), 7.60 - 7.64 (m, 3H, H-3', H-4' and H-5'), 5.78 (brs, 2H, CH_2-exocyclic), 11.74 (brs, 1H, NH-benzyl), 4.85 (s, 2H, CH_2-benzyl), 7.31-7.38 (m, 3H, CH-benzyl), 7.42 (d, *J* 3.6 Hz, 2H, CH-benzyl). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 141.6 (C-1), 138.6 (C-3), 115.9 (C-4), 129.9 (C-4a), 121.0 (C-4b), 122.2 (C-5), 120.4 (C-6), 129.1 (C-7), 112.7 (C-8), 140.4 (C-8a), 137.4 (C-9a), 133.9 (C-1'), 128.4 (C-2'/6'), 129.0 (C-3'/5'), 128.9 (C-4'), 175.1 (C-2"), 160.1 (C-5"), 67.7 (CH_2-exocyclic), 47.7 (CH_2-benzyl), 135.4 (C-benzyl), 128.2 (CH-benzyl), 128.8 (CH-benzyl), 128.0 (CH-benzyl). EIMS, 70 eV, *m/z* (rel. int., %): 463 (10, $M^{+\cdot}$), 344 (95), 242 (45), 191 (35), 91 (100).

1-(3-Nitrophenyl)-3-[3-benzylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-*carboline (**5b**)*

Yield: 77%, mp 216.0-220.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3063 (N–H), 1621 (C=N), 1348 and 1240 (C=S), 1566, 1496 and 1454 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.98 (s, 1H, H-4), 8.48 (d, *J* 7.5 Hz, 1H, H-5), 7.30-7.40 (m, 1H, H-6), 7.62 - 7.71 (m, 2H, H-7 and H-8), 12.14 (s, 1H, 9-NH), 8.71 (brs, 1H, H-2'), 8.43 (dd, *J* 7.2 and 1.8 Hz, 1H, H-4'), 7.91 (t, *J* 7.2 Hz, 1H, H-5'), 8.32 (d, *J* 7.2 Hz, 1H, H-6'), 5.79 (brs, 2H, CH_2-exocyclic), 11.78 (brs, 1H, NH-benzyl), 4.82 (s, 2H, CH_2-benzyl), 7.29-7.40 (m, 5H, CH-benzyl). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 142.1 (C-1), 138.8 (C-3), 116.7 (C- 4), 127.8 (C-4a), 121.0 (C-4b), 122.4 (C-5), 120.7 (C-6), 129.2 (C-7), 112.6 (C-8), 141.6 (C-8a), 134.8 (C-9a), 134.1 (C-1'), 123.2 (C-2'), 148.2 (C-3'), 123.6 (C-4'), 130.4 (C-5'), 135.4 (C-6'), 176.4 (C-2"), 160.2 (C-5"), 67.8 (CH_2-exocyclic), 47.7 (CH_2-benzyl), 137.9 (C-benzyl), 128.0 (CH-benzyl), 128.6 (CH-benzyl), 127.8 (CH-benzyl). EIMS, 70 eV, *m/z* (rel. int., %): 509 (5, $M^{+\cdot}$), 191 (10), 91 (100), 65 (15).

1-(4-N,N-Dimethylaminophenyl)-3-[3-benzylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-*carboline (**5c**)*

Yield: 86%, mp 200.0-205.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3086 (N–H), 1607 (C=N), 1363 and 1241 (C=S), 1558, 1495 and 1443 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.79 (s, 1H, H-4), 8.40 (d, *J* 7.8 Hz, 1H, H-5), 7.28-7.39 (m, 1H, H-6), 7.59 (t, *J* 7.8 Hz, 1H, H-7), 7.68-7.76 (m, 3H, H-8, H-2' and H-6'), 11.84 (s, 1H, 9-NH), 6.86 (d, *J* 8.4 Hz, 2H, H-3' and H-5'), 3.06 (s, 6H, $N(CH_3)_2$), 5.80 (brs, 2H, CH_2-exocyclic), 4.86 (s, 2H, CH_2-benzyl), 7.43 (d, *J* 4.5 Hz, CH-benzyl), 7.28-7.39 (m, 3H, CH-benzyl). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 141.4 (C-1), 135.4 (C-3), 114.7 (C-4), 124.8 (C-4a), 121.2 (C-4b), 122.0 (C-5), 120.3 (C-6), 128.5 (C-7), 112.8 (C-8), 141.1 (C-8a), 133.4 (C-9a), 129.3 (C-1'), 129.1 (C- 2'/6'), 112.1 (C-3'/5'), 150.7 (C-4'), 175.1 (C-2"), 160.1 (C-5"), 40.0 $-N(CH_3)_2$, 67.8 (CH_2-exocyclic), 47.8 (CH_2-benzyl), 138.4 (C-benzyl), 128.8 (CH-benzyl), 128.2 (CH-benzyl), 127.9 (CH-benzyl). EIMS, 70 eV, *m/z* (rel. int., %): 506 (5, $M^{+\cdot}$), 242 (45), 191 (35), 91 (100).

1-Phenyl-3-[3-pyrrolidylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-*carboline (**6a**)*

Yield: 55%, mp 207.0-209.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3280 (N-H), 1622 (C=N), 1374 and 1243 (C=S), 1567, 1496, 1445 and 1415 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.87 (s, 1H, H-4), 8.50 (d, *J* 7.8 Hz, 1H, H-5), 7.35 (t, *J* 7.8 Hz, 1H, H-6), 7.57 - 7.72 (m, H-7, H-8, H-3', H- 4' and H-5'), 11.99 (brs, 1H, 9-NH), 8.05 (d, *J* 6.9 Hz, 2H, H-2' and H-6'), 5.20 (brs, 2H, CH_2-exocyclic), 2.89 (brs, 4H, CH_2-pyrrolidyl), 1.71 (brs, 4H, CH_2-pyrrolidyl).^{13}C NMR (75.5 MHz, DMSO-d_6): δ 142.8 (C-1), 137.2 (C-3), 113.9 (C-4), 120.9 (C-4b), 122.4 (C-5), 120.5 (C-6), 129.5 (C-7), 112.8 (C-8), 141.6 (C-8a), 134.0 (C-9a), 131.1 (C-1'), 128.6 (C-2'/6'), 128.9 (C-3'/5'), 129.2 (C-4'), 177.8 (C-2"), 159.5 (C-5"), 66.1 (CH_2-exocyclic), 49.5 (CH_2-pyrrolidyl), 23.6 (CH_2-pyrrolidyl). EIMS, 70 eV, *m/z* (rel. int., %): 427 (5, $M^{+\cdot}$), 243 (100), 189 (10), 121 (40).

1-(3-Nitrophenyl)-3-[3-pyrrolidylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-*carboline (**6b**)*

Yield: 52%, mp 112.0-114.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3285 (N-H), 1623 (C=N), 1347 and 1252 (C=S), 1523,

1498 and 1455 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.95 (s, 1H, H-4), 8.54 (d, *J* 7.8 Hz, 1H, H-5), 7.37 (t, *J* 7.8 Hz, 1H, H-6), 7.63-7.72 (m, 2H, H-7 and H-8), 12.17 (s, 1H, 9-NH), 8.80 (s, 1H, H-2'), 8.50 (d, *J* 7.8 Hz, 1H, H-4'), 7.96 (t, *J* 7.8 Hz, 1H, H-5'), 8.44 (d, *J* 7.8 Hz, 1H, H-6'), 5.20 (brs, 2H, CH_2-exocyclic), 2.89 (brs, 4H, CH_2-pyrrolidyl), 1.71 (brs, 4H, CH_2-pyrrolidyl). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 141.7 (C-1), 138.6 (C-3), 130.1 (C-4), 120.9 (C-4b), 122.5 (C-5), 120.7 (C-6), 129.3 (C-7), 112.7 (C-8), 140.2 (C-8a), 134.1 (C-9a), 131.4 (C-1'), 123.4 (C-2'), 148.2 (C-3'), 123.7 (C-4'), 130.6 (C-5'), 135.1 (C-6'), 177.9 (C-2"),159.3 (C-5"), 66.2 (CH_2-exocyclic), 49.5 (CH_2-pyrrolidyl), 23.6 (CH_2-pyrrolidyl). EIMS, 70 eV, *m/z* (rel. int., %): 472 (10, M^+), 289 (70), 188 (10), 121 (25), 70 (100).

1-(4-N,N-Dimethylaminophenyl)-3-[3-pyrrolidylamino (methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-*carboline (**6c**)*

Yield: 92%, mp 156.0-160.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3080 (N-H), 1623 (C=N), 1369 and 1243 (C=S), 1557, 1497 and 1455 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.74 (s, 1H, H-4), 8.46 (d, *J* 8.1 Hz, 1H, H-5), 7.32 (t, *J* 8.1 Hz, 1H, H-6), 7.60 (t, *J* 8.1 Hz, 1H, H-7), 7.71 (d, *J* 8.1, 1H, H-8), 11.87 (s, 1H, 9-NH), 7.96 (d, *J* 9.0 Hz, 1H, H-2'), 6.97 (d, *J* 9.0 Hz, 1H, H-3'), 6.96 (d, *J* 9.0 Hz, 1H, H-5'), 7.96 (d, *J* 9.0 Hz, 1H, H-6'), 3.05 (s, 1H, $N(CH_3)_2$), 5.20 (brs, 2H, CH_2-exocyclic), 2.89 (brs, 4H, CH_2-pyrrolidyl), 1.71 (brs, 4H, CH_2-pyrrolidyl). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 143.5 (C-1), 133.5 (C-3), 112.7 (C-4), 124.6 (C-4a), 121.0 (C-4b), 122.1 (C-5), 120.3 (C-6), 128.6 (C-7), 112.8 (C-8), 141.4 (C-8a), 130.8 (C-9a), 129.0 (C-1'), 129.4 (C-2'), 112.1 (C-3'), 150.9 (C-4'), 112.1 (C-5'), 129.4 (C-6'), 177.6 (C-2"), 163.7 (C-5"), 40.0 –$N(CH_3)_2$, 66.1 (CH_2-exocyclic), 49.5 (CH_2-pyrrolidyl), 23.6 (CH_2-pyrrolidyl). EIMS, 70 eV, *m/z* (rel. int., %): 470 (10, M^+), 287 (100), 120 (35).

1-Phenyl-3-[3-morpholylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-*carboline (**7a**)*

Yield: 82%, mp 208.0-210.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3207 (N-H indol), 1622 (C=N), 1359 and 1248 (C=S), 1561, 1496 and 1455 cm^{-1} (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.90 (s, 1H, H-4), 8.52 (d, *J* 7.8 Hz, 1H, H-5), 7.35 (t, *J* 7.8, 1H, H-6), 7.58-7.73 (m, 5H, H-7, H-8, H-3', H-4' and H-5'), 12.03 (s, 1H, 9-NH), 8.05 (d, *J* 6.9 Hz, 1H, H-2' and H-6'), 5.08 (brs, 2H, CH_2-exocyclic), 2.81 (t, *J* 4.3 Hz, 4H, CH_2-morpholyl), 3.61 (t, *J* 4.3 Hz, 4H, CH_2-morpholyl). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 143.0 (C-1), 137.2 (C-3), 114.3 (C-4), 129.0 (C-4a), 120.9 (C-4b), 122.4 (C-5), 120.5 (C-6), 129.5 (C-7), 112.8 (C-8), 141.6 (C-8a), 134.1 (C-9a), 130.6 (C-1'), 128.6 (C-2'/6'), 128.9 (C-3'/5'), 129.3 (C-4'), 177.6 (C-2"), 161.2 (C-5"), 70.0 (CH_2-exocyclic), 50.1 (CH_2-morpholyl), 66.4 (CH_2-morpholyl). EIMS, 70 eV, *m/z* (rel. int., %): 443 (5, M^+), 243 (100), 121 (20), 58 (25).

1-(3-Nitrophenyl)-3-[3-morpholylamino(methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-*carboline (**7b**)*

Yield: 85%, mp 163.0-167.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3226 (N-H), 1626 (C=N), 1351 and 1249 (C=S), 1559, 1497 and 1453 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.98 (s, 1H, H-4), 8.55 (d, *J* 7.8 Hz, 1H, H-5), 7.38 (t, *J* 7.8 Hz, 1H, H-6), 7.64-7.73 (m, 2H, H-7, H-8), 12.21 (s, 1H, 9-NH), 8.80 (s, 1H, H-2'), 8.50 (d, *J* 7.8 Hz, 1H, H-4'), 7.97 (t, *J* 7.8 Hz, 1H, H-5'), 8.45 (dd, *J* 7.8 and 1.8 Hz, 2H, H-6'), 5.09 (brs, 2H, CH_2-exocyclic), 2.81 (brs, 4H, CH_2-morpholyl), 3.61 (brs, CH_2-morpholyl). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 141.7 (C-1), 148.2 (C-3), 115.0 (C-4), 122.6 (C-5), 120.8 (C-6), 129.4 (C-7), 112.7 (C-8), 140.4 (C-8a), 134.3 (C-9a), 130.1 (C-1'), 123.4 (C-2'), 148.2 (C-3'), 123.8 (C-4'), 130.6 (C-5'), 135.1 (C-6'), 177.7 (C-2"), 160.6 (C-5"), 70.0 (CH_2-exocyclic), 50.1 (CH_2-morpholyl), 66.0 (CH_2-morpholyl).EIMS, 70 eV, *m/z* (rel. int., %): 488 (5, M^+), 289 (95), 242 (100), 121 (20), 57 (25).

1-(4-N,N-Dimethylaminophenyl)-3-[3-morpholylamino (methyl)-2-thioxo-1,3,4-oxadiazol-5-yl] β-*carboline (**7c**)*

Yield: 83%, mp 215.0-217.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3261 (N-H), 1609 (C=N), 1364 and 1238 (C=S), 1556, 1495 and 1438 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.75 (s, 1H, H-4), 8.46 (d, *J* 8.1 Hz, 1H, H-5), 7.33 (t, *J* 8.1 Hz, 1H, H-6), 7.60 (t, *J* 8.1 Hz, H-7), 7.72 (d, *J* 8.1 Hz, 1H, H-8), 11.90 (s, 1H, 9-NH), 7.96 (d, *J* 8.7 Hz, 1H, H-2'), 6.97 (d, *J* 8.7 Hz, 1H, H-3'), 6.97 (d, *J* 8.7 Hz, 1H, H-5'), 7.96 (d, *J* 8.7 Hz, 1H, H-6'), 3.05 (s, 6H, $N(CH_3)_2$), 5.08 (brs, 2H, CH_2-exocyclic), 2.81 (brs, 4H, CH_2-morpholyl), 3.61 (brs, CH_2-morpholyl). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 143.6 (C-1), 133.6 (C-3), 112.9 (C-4), 124.5 (C-4a), 121.0 (C-4b), 122.1 (C-5), 120.4 (C-6), 128.6 (C-7), 112.8 (C-8), 141.4 (C-8a), 130.7 (C-9a), 128.9 (C-1'), 129.4 (C-2'), 112.1 (C-3'), 150.9 (C-4'), 112.1 (C-5'), 129.4 (C-6'), 177.6 (C-2"), 161.4 (C-5"), 39.9 - $N(CH_3)_2$, 69.9 (CH_2-exocyclic), 50.1 (CH_2-morpholyl), 66.0 (CH_2-morpholyl). EIMS, 70 eV, *m/z* (rel. int., %): 486 (5, M^+), 242 (30), 121 (15), 57 (100).

Anticancer assays

The synthesized compounds were evaluated *in vitro* against eight-cell panel lines consisting of melanoma (UACC-62), breast (MCF7), ovarian resistant (NCI/ADR), renal (786-0), lung (NCI-460), prostate (PCO-3), ovarian

(OVCAR) and colon (HT-29). The tests were performed by the colorimetric method with sulforodamine B, according to NCI standard protocol and doxorubicin was used as positive control.[22] Assays were performed in a 96-well plate using four concentrations at 10-fold dilutions (0.25 mg mL^{-1} to 250 mg mL^{-1}) for each test compound. The anticancer activity was deduced from concentration-response curves and three concentration response parameters (GI_{50}, TGI and LC_{50}) were calculated. The GI_{50} values (growth inhibitory activity) (Table 1) refer to the drug concentration that produce 50% reduction of cellular growth when compared to untreated control cells.[23] The TGI (cytostatic activity) and LC_{50} (cytotoxic activity) values parameters (Table 2) refer to the drug concentration for total growth inhibition and for killing 50% of cells, respectively. Compounds with GI_{50} values < 100 μmol L^{-1} were considered active. A mean graph midpoint values (MG-MID) of the compounds toward tumor cell lines were calculated for each of the parameters GI_{50}, TGI and LC_{50}. MG-MID values supplied an averaged activity parameter for all cell lines.[23]

Antimicrobial activity

The bacterial strains were grown overnight at 36 °C in Nutrient Agar (Merck), and the strains were grown in Saboraud 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).[24] 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 24h. Antibacterial activity was detected by adding 20 μL of 0.5% TTC (triphenyl tetrazolium chloride, Merck) aqueous solution. 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.[25]

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Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br, as PDF file.

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