Synthesis, Antitumor, Antitrypanosomal and Antileishmanial Activities of Benzo[4,5]canthin-6-ones Bearing the *N'*-(Substituted benzylidene)-carbohydrazide and *N*-Alkylcarboxamide Groups at C-2

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A series of novel benzo[4,5]canthin-6-ones, bearing the *N'*-(substituted benzylidene)-carbohydrazide (11a–e) and *N*-alkylcarboxamide (13a–g) moieties at position-2, were synthesized and screened for their *in vitro* antitumor activity, against seven human cancer cell lines, and for antitrypanosomal and antileishmanial activities against *Trypanosoma cruzi* and *Leishmania amazonensis*. The results indicated that *N*-methylpiperazyl-6-oxobenzo[4,5]canthine-2-carboxamide (13f) displayed potent antitumor activity with IC₅₀ values in the range of 1.15–8.46 μM for all cell lines tested. Compounds 13f and 13g bearing an *N*-methylpiperazylcarboxamide and *N*-morpholylcarboxamide at C-2, respectively, showed potent activities towards both *Trypanosoma cruzi* and *Leishmania amazonensis* parasites, with IC₅₀ in the range of 0.4 to 16.70 μM.

Key words benzocanthinone; benzylidenecarbohydrazide; carboxamide; antitumor activity; antitrypanosomal activity; antileishmanial activity

Canthin-6-ones are a subclass of β-carboline alkaloids, with an additional D-ring, which have been found in plants of different families, displaying important biological activities, such as anti-ulcer,¹⁾ anti-inflammatory,²⁾ anti-human immunodeficiency virus (HIV),³⁾ trypanocidal and leishmanicidal,^{4,5)} antimicrobial^{6–8)} and cytotoxic.^{9–11)} *In vivo* leishmanicidal and trypanocidal activities of canthin-6-ones (1, Fig. 1) isolated of *Zanthoxylum chiloperone* (Rutaceae) were observed in Balb/c mice infected with *Leishmania amazonensis* and *Trypanosoma cruzi*, respectively.^{4,5)} Antifungal activity similar to those of positive control ketoconazole, against pathogenic fungi, were reported for natural and synthetic canthin-6-one derivatives.⁶⁾ Canthin-6-one-*N*-oxide and benzo[4,5]canthin-6-one derivatives (2, Fig. 1) were active towards different type of *Mycobacterium* species, such as *Mycobacterium tuberculosis* and *M. bovis*. These compounds were patented as medicaments for tuberculosis and Hansen's disease treatment.⁷⁾ Antitumor activity of 1-methoxycanthin-6-one, isolated from *Ailanthus altissima* SWINGLE, were evaluated on apoptosis in human leukemia (Jurkat), thyroid carcinoma, and hepatocellular carcinoma (HuH7) cell lines. The study indicated that this compound can

represent a candidate for *in vivo* evaluation on monotherapies or combined antineoplastic therapies.⁸⁾ Canthin-6-one (1) and 9-methoxycanthin-6-one from roots of *Eurycoma longifolia* (Simaroubaceae) presented significant cytotoxicity against human lung (A-549) and human breast (MCF-7) cancer cell lines.⁹⁾ In another work with the same plant, the isolated canthin-6-ones were evaluated for their cytotoxic activity against a HT-1080 human fibrosarcoma cell lines. Compounds 9,10-dimethoxycanthin-6-one and 10-hydroxy-9-methoxycanthin-6-one displayed stronger activity than the positive control 5-FU (IC₅₀=9.2 μM).¹⁰⁾ Significant cytotoxic activity against CNE2 cell lines was reported for some canthin-6-ones isolated from the stem of *Picrasma quassioides* BENNET (Simaroubaceae).¹¹⁾ A recent work described the synthesis and cytotoxic activity evaluation of a series of novel 1,4-disubstituted and 1,4,9-trisubstituted β-carbolines and tetracyclic derivatives, which were designed on the basis of harmine and 1-methoxycanthin-6-one chemical structures.¹²⁾

In our previous studies on the structure–activity relationship of β-carboline alkaloids, we demonstrated that derivatives containing a *N'*-(substituted benzylidene)-carbohydrazide unit

Fig. 1. General Structures of Canthin-6-ones (1), Benzo[4,5]canthin-6-ones (2) and 1-(Substituted phenyl)-3-substituted-β-carbolines (3 and 4)

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Chart 1. Synthetic Route for Methyl 6-Oxobenzo[4,5]canthine-2-carboxylate (**9**) Chart 2. Synthesis of *N'*-(Substituted benzylidene)-6-oxobenzo[4,5]canthine-2-carbohydrazides (**11a–e**)

at C-3 (**3**, Fig. 1), presented significant antitumor activities, with IC_{50} values lower than $10\mu M$ against a panel of human cancer cell lines.¹³ Additionally, 1-(substituted phenyl)- β -carboline bearing an *N*-alkylcarboxamide group at position-3 (**4**, Fig. 1) displayed significant antileishmanial and antitrypanosomal activities against *Leishmania amazonensis* and *Trypanosoma cruzi*, respectively.^{14–17} These observations, associated with the wide range of biological activities of canthin-6-one derivatives, and in continuing our studies on novel antitumor and antiprotozoal agents, in this work we synthesized and evaluated the antitumor, antitrypanosomal and antileishmanial activities of new series of benzo[4,5]canthin-6-ones bearing a *N'*-(substituted benzylidene)-carbohydrazide (**11a–e**) and *N*-alkylcarboxamide (**13a–g**) groups at C-2.

Results and Discussion

Synthesis The approaches for the synthesis of compounds **11a–e** and **13a–g** are outlined in Charts 1, 2 and 3. The synthetic work was conducted in order to compare the activities of the conformationally restricted benzo[4,5]canthin-6-one nucleus with those for previously reported 1-(substituted phenyl)- β -carboline,^{13,16,17} having the rotational freedom at the 1-substituted-phenyl group. For this propose, beside others, 2-substituted benzo[4,5]canthin-6-one derivatives, with the same substitution pattern on the benzylidene-carbohydrazide and carboxamide groups that those presented for the most active 1-(substituted phenyl)- β -carboline analogues were synthesized. The synthesis of derivatives **11a–e** and **13a–g** were carried out by employing the procedure reported by Soriano-Agatón *et al.*,⁶ with some modifications. To access the desired 2-substituted benzo[4,5]canthin-6-one derivatives, the methyl 6-oxobenzo[4,5]canthine-2-carboxylate (**9**) was used as the key intermediate, using L-tryptophan (**1**) as starting material (Chart 1). Condensation of L-tryptophan methyl ester (**6**) with

phthalic anhydride in CH_2Cl_2 at room temperature afforded the amide **7**. A modified Bischler–Napieralski cyclization of amide **7**, employing the Vilsmeier reagent ($SOCl_2/DMF$), led to the acid chloride **8**, which without isolation was treated with DBU in CH_2Cl_2 at room temperature, to give the methyl 6-oxobenzo[4,5]canthine-2-carboxylate (**9**). Oxidation of the C-1/C-2 bond occurred spontaneously, as reported previously by Soriano-Agatón *et al.*⁶

The structure of compound **9** was confirmed by IR, ^1H - and ^{13}C -NMR and MS spectral data. IR spectrum showed absorption bands at 1721 and 1697 cm^{-1} , relative to the ester and α,β -unsaturated ketone moieties, respectively. ^1H -NMR spectrum revealed signals for the β -carboline nucleus at δ 8.87 (s, H-1), 8.81 (d, $J=7.8\text{ Hz}$, H-8), 7.83–7.75 (m, H-9), 7.59 (td, $J=7.8$, 0.9 Hz, H-10) and 8.21 (d, $J=7.8\text{ Hz}$, H-11). The additional D-ring was evidenced by signals at δ 8.96 (dd, $J=7.8$, 0.9 Hz, H-12), 7.95 (td, $J=7.8$, 0.9 Hz, H-13), 7.83–7.75 (m, H-14), 8.66 (dd, $J=7.8$, 0.9 Hz, H-15). The signals at δ 166.3 and 159.7 in the ^{13}C -NMR spectrum were assigned to the carbonyl of the carbomethoxy and α,β -unsaturated ketone groups, respectively.

The synthetic route for conversion of intermediate **9** to the desired *N'*-(substituted benzylidene)-6-oxobenzo[4,5]canthine-2-carbohydrazides (**11a–e**) is outlined in Chart 2. Reaction of the ester **9** with hydrazine hydrate, followed by reaction of the resulting 6-oxobenzo[4,5]canthine-2-carbohydrazide (**10**) with aromatic aldehydes bearing electron-withdrawing and electron-donating groups, under acid catalysis with sulfuric acid, afforded the carbohydrazides **11a–e**. The 6-oxobenzo[4,5]canthine derivatives **11a–d** contains the same substitution pattern to that of most active *N'*-(substituted benzylidene)-1-(substituted phenyl)- β -carboline-3-carbohydrazides previously reported (compounds of **3a–d** series shown in Table 1).¹³

Chart 3. Synthesis of *N*-Alkyl-6-oxobenzo[4,5]canthine-2-carboxamides (**13a–g**)

The compounds synthesized were characterized with basis on their ^1H - and ^{13}C -NMR, IR and EM spectroscopic data. ^1H -NMR spectra of **11a–e** showed two singlets at δ 8.65–9.14 (s, 1H) and 11.95–12.55 (s, 1H) due to imine ($-\text{CH}=\text{N}-$) and amide ($-\text{CONH}-$) protons, respectively, in addition to the proton signals of the respective substituted phenyl groups of *N'*-(substituted benzylidene)-carbohydrazide moiety. In the ^{13}C -NMR spectra, the carbons of $\text{C}=\text{N}-$ and $\text{CONH}-$ groups appeared at δ 144.9–150.9 and 159.8–161.0, respectively. Electron impact (EI)-MS spectra showed molecular ions consistent with the structures of synthesized compounds **11a–e**.

To prepare the *N*-alkyl-6-oxobenzo[4,5]canthine-2-carboxamides (**13a–g**), compound **9** was hydrolyzed with a mixture of AcOH and HCl to afford the corresponding carboxylic acid **12** (Chart 3). The hydrolysis of the ester group was confirmed by absence of the ester methyl protons at δ 3.59, in the ^1H -NMR spectrum, and the presence of a signal at δ 166.1 due the carbonyl of carboxylic acid in the ^{13}C -NMR spectrum. Compound **12** was converted to its acid chloride by using thionyl chloride. Treatment of the crude product with different primary or secondary amines resulted in the *N*-alkyl-6-oxobenzo[4,5]canthine-2-carboxamides (**13a–g**). Compounds **13a–e** possess the same *N*-alkylcarboxamide groups present in the most active β -carboline-3-*N*-alkylcarboxamides from

our previous work (compounds of **4a–e** series shown in Table 3).^{16,17)}

The characterization of compounds **13a–g** was based on their IR, EI-MS and NMR spectra data.

In Vitro Antitumor Activity The newly synthesized *N'*-(substituted benzylidene)-6-oxobenzo[4,5]canthine-2-carbohydrazides (**11a–e**) and *N*-alkyl-6-oxobenzo[4,5]canthine-2-carboxamides (**13a–g**) were evaluated for their *in vitro* antitumor activities against seven human cancer cell lines. Compounds of the *N'*-(substituted benzylidene)-carbohydrazide series **11a–e** were inactive against all human cancer cell lines tested, displaying IC_{50} values higher than $100\ \mu\text{M}$. The lack of activity of compounds **11a–d**, in comparison with the 1-(substituted phenyl)- β -carboline previously synthesized, bearing the same benzylidene-carbohydrazide groups (compounds of **3a–d** series in Table 1)¹³⁾ suggests that the 1-(substituted phenyl)- β -carboline is a better pharmacophore for cytotoxic activity than the conformationally constrained benzo[4,5]-canthin-6-one nucleus.

Compounds of the *N*-alkylcarboxamide series, except **13b**, **13c** and **13e**, exhibited moderated to significant activity (Table 2), showing that the *N*-alkylcarboxamide moiety at C-2 is a promising pharmacophore for cytotoxic activity of benzo[4,5]canthin-6-ones. It is worth to mention the derivative

Table 1. *In Vitro* Antitumor Activity Data of Benzylidene-Carbohydrazides of **3a–d** Series and of **11a–e**

Compound	R	R ¹	IC ₅₀ values against cell lines tested						
			U251 glioma	MCF7 breast	NCI/ADR-Res. ovarian	786-0 renal	NCI-H460 lung	OVCAR-3 ovarian	HT29 colon
Dox ^{a)}	—	—	0.05	0.08	0.23	0.08	0.02	0.16	0.37
3a₁	Ph	3-NO ₂	nt	2.61	nt	3.76	1.93	6.14	1.93
3a₂	Ph	4-OH	nt	8.94	4.32	4.32	19.83	1.26	4.32
3b	4-OMe-Ph	4-NO ₂	nt	8.19	nt	14.09	4.58	21.40	8.92
3c₁	2-Cl-Ph	4-OH	nt	7.01	2.6	3.22	2.18	1.83	1.71
3c₂	2-Cl-Ph	4-OMe	nt	10.38	2.19	2.52	1.43	7.43	1.26
3c₃	2-Cl-Ph	H	nt	6.13	19.4	2.16	4.57	2.84	1.25
3d	4-(Me ₂)NPh	3-NO ₂	nt	0.63	0.05	0.04	2.11	0.19	9.80
11a	Ph	—	>100	>100	>100	>100	>100	>100	>100
11b	4-OMe-Ph	—	>100	>100	>100	>100	>100	>100	>100
11c	2-Cl-Ph	—	>100	>100	>100	>100	>100	>100	>100
11d	4-(Me ₂)NPh	—	>100	>100	>100	>100	>100	>100	>100
11e	4-F-Ph	—	>100	>100	>100	>100	>100	>100	>100

a) Doxorubicin was used as positive control; nt=not tested.

13f, containing the *N*-methylpiperazylcarbohydrazide at C-2, which displayed a high activity with IC_{50} values in the range of 1.15–8.46 μM for all cell lines tested. The highest cytotoxicity was observed towards ovarian cancer cell lines (OVCAR-3) with IC_{50} of 1.15 μM . Compound **13d**, bearing a *N*-cyclohexylcarboxamide group at C-2, showed high selectivity for ovarian cancer cell lines (OVCAR-3) with IC_{50} values of 11.04 μM . Introduction of the *N*-butyl-, *N*-pyrrolidyl- and *N*-benzyl-carboxamide groups at C-2 resulted in loss of cytotoxic activity as shown for compounds **13b**, **13c** and **13e**, respectively, with IC_{50} values $\geq 100 \mu M$ toward all cell lines tested.

The antitumor mode of action of β -carboline alkaloids has been widely investigated. Several studies has pointed that the interaction with DNA, enzymatic or receptor systems are the most commonly mode of action observed for antitumor compounds of this class.¹⁸⁾ Also, it was demonstrated that the position-1 is a important site for antitumor activity of β -carboline derivatives; appropriate substituent at this position can enhance their antitumor activity.¹⁸⁾ On the other hand, no

study on the mechanism of action of benzo[4,5]canthin-6-one derivatives is reported.

Antitrypanosomal and Antileishmanial Activities The synthesized compounds **11a–e** and **13a–g** were evaluated for their activities against promastigote forms of *Leishmania amazonensis* and epimastigote forms of *Trypanosoma cruzi*. The assays results demonstrated that compounds of *N'*-(substituted benzylidene)-carbohydrazide series (**11a–e**) were inactive against both parasites, displaying $IC_{50} \geq 100 \mu M$. The IC_{50} data of the newly synthesized compounds **13a–g** and of the most active 1-(phenyl substituted)- β -carbolines (**4a–e** series)^{16,17)} are shown in Table 3.

In general, the assays data indicated that epimastigote forms of *Trypanosoma cruzi* were more susceptible towards the tested compounds than promastigote forms of *Leishmania amazonensis*. The *N*-alkylcarboxamides **13a–e** displayed lower antiprotozoal activity ($IC_{50} > 50 \mu M$) in comparison to the *N*-alkyl-1-(substituted phenyl)- β -carboline-3-carboxamides (**4a–e** series in Table 3) bearing the same alkyl groups,^{16,17)}

Table 2. *In Vitro* Antitumor Activity Data of Compounds **13a–g**

Compound	IC_{50} values against cell lines tested						
	U251 glioma	MCF7 breast	NCI/ADR-Res. ovarian	786-0 renal	NCI-H460 lung	OVCAR-3 ovarian	HT29 colon
Doxorubicin ^{a)}	0.05	0.08	0.23	0.08	0.02	0.16	0.37
13a	76.26	84.15	69.77	79.36	69.71	98.20	74.67
13b	>100	>100	>100	>100	>100	>100	>100
13c	>100	>100	>100	>100	>100	>100	>100
13d	79.55	73.20	67.52	70.12	83.26	11.04	>100
13e	>100	>100	70.90	>100	>100	>100	>100
13f	6.34	8.46	2.62	6.86	5.70	1.15	5.19
13g	62.26	68.34	20.29	67.35	34.67	73.90	66.28

a) Doxorubicin was used as positive control.

Table 3. Antitrypanosomal and Antileishmanial Assays Data for Compounds of **4a–e** Series and for **13a–g**

	R	R ¹	IC_{50} (μM)	
			<i>L. amazonensis</i>	<i>T. cruzi</i>
4a₁	<i>i</i> -Propyl	H	7.00 $\pm$ 1.30	9.71 $\pm$ 1.70
4a₂	<i>i</i> -Propyl	4-OMe	5.30 $\pm$ 0.30	6.40 $\pm$ 0.84
4a₃	<i>i</i> -Propyl	3-OMe-4-OH	4.68 $\pm$ 0.37	6.66 $\pm$ 1.04
4b₁	<i>n</i> -Butyl	H	8.80 $\pm$ 2.00	14.20 $\pm$ 1.97
4b₂	<i>n</i> -Butyl	4-OMe	0.25 $\pm$ 0.07	3.22 $\pm$ 0.50
4b₃	<i>n</i> -Butyl	2-Cl	2.45 $\pm$ 0.91	5.20 $\pm$ 0.28
4c₁	Pyrrolidyl	H	8.90 $\pm$ 1.34	8.10 $\pm$ 0.42
4c₂	Pyrrolidyl	4-N(Me) ₂	2.42 $\pm$ 0.41	7.97 $\pm$ 0.23
4c₃	Pyrrolidyl	2-Cl	4.43 $\pm$ 0.39	8.63 $\pm$ 0.79
4d₁	Cyclohexyl	H	21.6 $\pm$ 2.40	4.39 $\pm$ 0.54
4d₂	Cyclohexyl	4-OMe	3.60 $\pm$ 0.50	5.76 $\pm$ 0.84
4d₃	Cyclohexyl	2-Cl	3.57 $\pm$ 0.64	11.10 $\pm$ 1.77
4e₁	Benzyl	H	3.88 $\pm$ 0.78	7.42 $\pm$ 0.94
4e₂	Benzyl	2-Cl	5.12 $\pm$ 0.75	12.9 $\pm$ 1.70
13a	<i>i</i> -Propyl	—	>100	68.98 $\pm$ 2.12
13b	<i>n</i> -Butyl	—	>100	74.49 $\pm$ 9.19
13c	Pyrrolidyl	—	>100	>100
13d	Cyclohexyl	—	>100	54.43 $\pm$ 4.95
13e	Benzyl	—	>100	>100
13f	<i>N</i> -Methylpiperazyl	—	0.90 $\pm$ 0.13	0.40 $\pm$ 0.01
13g	Morpholyl	—	14.87 $\pm$ 2.36	16.70 $\pm$ 1.27

indicating that the 1-(substituted phenyl)- β -carboline is a better scaffold for antiprotozoal activity than the benzo[4,5]-canthin-6-one nucleus. Corroborating our conclusion, a recent study also reported a potent anti-leishmanial activity for some 1-aryl- β -carboline derivatives against *Leishmania donovani*.¹⁹⁾

On the other hand, compounds **13f** and **13g**, bearing *N*-methylpiperazylcarboxamide and *N*-morpholylcarboxamide groups at C-2, respectively, showed moderated to high activities towards both parasites. Compound **13f** was remarkably active, displaying IC_{50} of $0.90 \pm 0.13 \mu M$ and $0.40 \pm 0.01 \mu M$ towards *Leishmania amazonensis* and *Trypanosoma cruzi*, respectively, indicating that the *N*-methylpiperazylcarboxamide group might play a crucial role in enhancing the antiprotozoal activity.

The mechanisms of action of β -carbolines and canthin-6-ones as trypanocidal and leishmanicidal agents are not well established. Rivas *et al.*²⁰⁾ suggested that associations with flavoenzymes of the parasites, belonging or not to the respiratory chain, and/or alterations of parasite DNA synthesis, may be responsible for the trypanocidal activity of β -carbolines. Fournet *et al.*²¹⁾ proposed that the trypanocidal activities of canthin-6-ones could be related to the inhibition of sterol C-14a demethylase in intracellular *Trypanosoma cruzi* amastigotes. Di Giorgio *et al.*²²⁾ demonstrated that antileishmanial mechanism of action of β -carbolines could involve interactions with DNA metabolism leading to an accumulation of parasites in the S-G2M phases of the cell-cycle or could result from the capacity of the molecule to prevent parasite internalization within macrophages by inhibiting *Leishmania* PKC activity.

In previous work we observed that the treatment of promastigote forms of *Leishmania amazonensis* with the most active compound from the *N*-alkyl-1-(substituted phenyl)- β -carboline-3-carboxamide series (*N*-benzyl-1-(4-methoxy)-phenyl-9*H*-beta-carboline-3-carboxamide) cause cell shape and number of flagella alterations, as well as, nuclear membrane damage.¹⁷⁾

Conclusion

In the present work we reported the synthesis and *in vitro* antitumor and antiprotozoal activities evaluation of two series of novel 2-substituted benzo[4,5]canthin-6-one derivatives. The compounds bearing the 2-*N'*-(substituted benzylidene)-carbohydrazide (**11a–e**) did not show any activity in both *in vitro* antitumor and antiprotozoal assays. Regarding the *N*-alkyl-6-oxobenzo[4,5]canthine-2-carboxamide series (**13a–g**), the derivative **13f** displayed a high cytotoxic activity against all cell lines tested, as well as, potent antiprotozoal activities towards both *Trypanosoma cruzi* and *Leishmania amazonensis* parasites, evidencing the importance of the *N*-methylpiperazylcarboxamide group for biological activity. Finally, our results demonstrated that the 1-substituted phenyl- β -carboline system is a better pharmacophore for both cytotoxic and antiprotozoal activities than the conformationally constrained benzo[4,5]canthin-6-one nucleus. This can be attributed to the fact that these nucleus are electronic and sterically distinct, which probably influenced their activities in a different mode, leading to a lower interaction with DNA, enzymatic systems or specific receptors for the benzo[4,5]canthin-6-one core compared to the 1-substituted phenyl- β -carboline core. However, a study focusing the mechanism of action of the synthesized compounds is necessary to confirm this hypothesis.

Experimental

Chemistry Melting points were determined in a Micro-Quimica MQAPF-301 apparatus and are uncorrected. ¹H and ¹³C spectra were recorded in a Varian spectrometer model Mercury plus 300 at 300MHz and 75.5MHz, respectively, with CDCl₃ and dimethyl sulfoxide (DMSO-*d*₆) as solvents and tetramethylsilane (TMS) as the internal standard. EI-MS spectra 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 compounds. All reagents were purchased from commercial suppliers.

Synthesis of Amide 7 To a solution of L-tryptophan methyl ester (**6**) (4.5mmol) in CH₂Cl₂ (30mL), was added phthalic anhydride (9mmol; 2eq). The mixture was stirred at room temperature for 5h. The solid was separated by filtration and washed with CH₂Cl₂ and recrystallized from methanol to afford compound **7**: amorphous solid, yield 82%; ¹H-NMR (DMSO-*d*₆) δ : 3.12–3.26 (m, 2H, H-8), 3.59 (s, 3H, OCH₃), 4.66 (q, 1H, H-9, *J*=7.2Hz), 6.99 (t, 1H, H-5, *J*=7.5Hz), 7.08 (t, 1H, H-6, *J*=7.5Hz), 7.35 (d, 2H, H-7, H-13, *J*=7.5Hz), 7.21 (s, 1H, H-2), 7.49–7.58 (m, 3H, H-4, H-14, H-15), 7.75 (d, 1H, H-16, *J*=7.5Hz), 8.85 (d, 1H, NH, *J*=7.5Hz), 10.86 (s, 1H, CONH), 12.84 (brs, 1H, COOH). ¹³C-NMR (DMSO-*d*₆) δ : 26.9 (C-8), 51.8 (OCH₃), 53.6 (C-9), 109.7 (C-3), 111.5 (C-7), 118.1 (C-4), 118.4 (C-5), 121.0 (C-6), 123.7 (C-2), 127.1 (C-3a), 127.8 (C-13), 129.1 (C-16), 129.5 (C-15), 131.0 (C-14), 131.1 (C-12), 136.1 (C-3b), 137.5 (C-17), 168.1 (C-18), 168.3 (C-11), 172.2 (C-19). IR (KBr) cm⁻¹: 3423 (NH), 3265 (OH), 1735 (OC=O), 1699 (NC=O).

Methyl 6-Oxobenzo[4,5]canthine-2-carboxylate (9) To a solution of the amide **7** (1.4mmol) in CHCl₃ was added SOCl₂ (16.4mmol, 12eq) and *N,N*-dimethylformamide (DMF) (1.4mmol, 1eq). The mixture was stirred at 0–5°C for 1h, and at room temperature for 18h. The solvent was removed under vacuum and the residue was dissolved in CH₂Cl₂ (20mL), followed by the addition of DBU (4.1mmol; 3eq). The mixture was stirred at room temperature for 24h. The organic layer was washed with water (4×10mL), dried over anhydrous Na₂SO₄ and filtrated. The solid obtained after solvent evaporation was recrystallized from MeOH to afford the product **9**: amorphous yellow solid, yield 65%; ¹H-NMR (CDCl₃) δ : 4.14 (s, 3H, OC–H₃), 7.59 (td, 1H, H-10, *J*=7.8, 0.9Hz), 7.75–7.83 (m, 2H, H-9, H-14), 7.95 (td, 1H, H-13, *J*=7.8, 0.9Hz), 8.21 (d, 1H, H-11, *J*=7.8Hz), 8.66 (dd, 1H, H-15, *J*=7.8, 0.9Hz), 8.81(d, 1H, H-8, *J*=7.8Hz), 8.96 (dd, 1H, H-12, *J*=7.8, 0.9Hz), 8.87 (s, 1H, H-1). ¹³C-NMR (CDCl₃) δ : 53.3 (OCH₃), 117.7 (C-1, C-8), 122.9 (C-11), 124.6 (C-12), 124.8 (C-11a), 125.9 (C-10), 129.5 (C-15), 129.6 (C-3a), 130.9 (C-14), 131.3 (C-9), 131.8 (C-3b), 134.0 (C-13), 134.3 (C-4, C-11b), 135.9 (C-5), 139.8 (C-7a), 143.7 (C-2), 159.7 (C-6), 166.3 (C-1'). IR (KBr) cm⁻¹: 1720 (OC=O), 1697 (NC=O).

General Procedure for the Synthesis of *N'*-(Substituted benzylidene)-6-oxobenzo[4,5]canthine-2-carbohydrazides (11a–e) To a solution of methyl 6-oxobenzo[4,5]canthine-2-carboxylate (**9**) (1.5mmol) in CHCl₃–MeOH 1:1 was added hydrazine hydrate 51% (30.5mmol, 20eq). The mixture was stirred under reflux for 72h and further, for 2h at 0°C for precipitation. The solid formed was separated by filtration and

washed with ethanol. The carbohydrazide **10** was obtained in 75% yield.

To a solution of 6-oxobenzo[4,5]canthine-2-carbohydrazide (**10**) (0.3 mmol) in water (10 mL) were added two drops of concentrated sulfuric acid. The mixture was kept under stirring at 65°C until complete dissolution. A solution of appropriate aldehyde (0.45 mmol, 1.5 eq) in ethanol (10 mL) was added and the mixture refluxed for 48 to 140 h. The mixture was placed into ice and neutralized with 10% aq. Na₂CO₃. The precipitate formed was filtered, washed with water and recrystallized from MeOH to give the *N'*-(substituted benzylidene)-6-oxobenzo[4,5]canthine-2-carbohydrazides (**11a–e**).

N'-Benzylidene-oxobenzo[4,5]canthine-2-carbohydrazide (**11a**): Yellow solid, yield 52%, mp 264.5–266.5°C; ¹H-NMR (DMSO-*d*₆) δ: 7.45–7.56 (m, 3H, H-3', H-4', H-5'), 7.64 (td, 1H, H-10, *J*=7.8, 1.2 Hz), 7.82 (td, 2H, H-2', H-6', *J*=7.8, 1.2 Hz), 7.90 (td, 2H, H-9, H-14, *J*=7.8, 1.2 Hz), 8.07 (td, 1H, H-13, *J*=7.8, 1.2 Hz), 8.51 (d, 1H, H-15, *J*=7.8 Hz), 8.59 (d, 1H, H-8, *J*=7.8 Hz), 8.64 (d, 1H, H-11, *J*=7.8 Hz), 8.84 (s, 1H, N=CH), 9.07 (s, 1H, H-1), 9.24 (d, 1H, H-12, *J*=7.8 Hz), 12.19 (s, 1H, NH). ¹³C-NMR (DMSO-*d*₆) δ: 115.6 (C-1), 116.5 (C-8), 123.3 (C-11a), 124.0 (C-11), 124.5 (C-12), 125.7 (C-10), 127.3 (C-2', C-6'), 128.7 (C-15), 129.2 (C-3a), 129.0 (C-3', C-5'), 130.3 (C-4'), 130.8 (C-14), 131.1 (C-3b), 131.3 (C-9), 131.6 (C-11b), 133.7 (C-4), 133.7 (C-13), 133.9 (C-1'), 134.4 (C-5), 139.1 (C-7a), 144.9 (C-2), 149.4 (HC=N), 158.8 (C-6), 160.4 (CONH). IR (KBr) cm⁻¹: 3303 (NH), 1679 (C=O). EI-MS, 70 eV, *m/z* (rel. int., %): 416.01 (4, M⁺), 270.01 (100), 313.01 (50).

N'-(4-Methoxybenzylidene)-6-oxobenzo[4,5]canthine-2-carbohydrazide (**11b**): Yellow solid, yield 44%, mp 250.2–252.6°C; ¹H-NMR (DMSO-*d*₆) δ: 3.85 (s, 3H, OCH₃), 7.08 (d, 2H, H-3', H-5', *J*=8.7 Hz), 7.63 (t, 1H, H-10, *J*=7.8 Hz), 7.82 (t, 1H, H-9, *J*=7.8 Hz), 7.77 (d, 2H, H-2', H-6', *J*=8.7 Hz), 7.89 (t, 1H, H-14, *J*=7.8 Hz), 8.08 (t, 1H, H-13, *J*=7.8 Hz), 8.50 (d, 1H, H-15, *J*=7.8 Hz), 8.59 (d, 1H, H-11, *J*=7.8 Hz), 8.63 (d, 1H, H-8, *J*=7.8 Hz), 8.76 (s, 1H, N=CH), 9.06 (s, 1H, H-1), 9.23 (d, 1H, H-12, *J*=7.8 Hz), 12.08 (s, 1H, N-H). ¹³C-NMR (DMSO-*d*₆) δ: 53.3 (OCH₃), 114.4 (C-3', C-5'), 115.4 (C-1), 116.6 (C-8), 124.0 (C-11), 124.5 (C-12), 125.7 (C-10), 126.9 (C-11a), 128.6 (C-15), 128.8 (C-2', C-6'), 129.2 (C-3a), 130.8 (C-14), 130.9 (C-9), 131.1 (C-3b), 131.4 (C-5), 131.5 (C-11b), 133.7 (C-4, C-1'), 133.8 (C-13), 139.1 (C-7a), 145.1 (C-2), 149.3 (HC=N), 158.8 (C-6), 160.1 (C-4'), 161.0 (CONH). IR (KBr) cm⁻¹: 1682 (C=O). EI-MS, 70 eV, *m/z* (rel. int., %): 446.02 (5, M⁺), 270.02 (100), 313.00 (53).

N'-(2-Chlorobenzylidene)-6-oxobenzo[4,5]canthine-2-carbohydrazide (**11c**): Yellow solid, yield 57%, mp >294°C decomp; ¹H-NMR (DMSO-*d*₆) δ: 7.49–7.52 (m, 2H, H-4', H-5'), 7.58–7.60 (m, 1H, H-6'), 7.66 (t, H-10, *J*=7.5 Hz), 7.86 (t, H-9, *J*=7.5 Hz), 7.93 (t, H-14, *J*=7.5 Hz), 8.11–8.17 (m, 2H, H-13, H-3'), 8.56 (d, 1H, H-15, *J*=7.5 Hz), 8.64 (d, 1H, H-11, *J*=7.5 Hz), 8.69 (d, 1H, H-8, *J*=7.5 Hz), 9.14 (s, 1H, H-1), 9.24 (s, 1H, N=CH), 9.32 (d, 1H, H-12, *J*=7.5 Hz), 12.55 (s, 1H, N-H). ¹³C-NMR (DMSO-*d*₆) δ: 115.9 (C-1), 116.6 (C-8), 121.5 (C-11a), 124.1 (C-11), 124.6 (C-12), 125.8 (C-10), 127.4 (C-2'), 127.8 (C-4'), 127.9 (C-3'), 128.8 (C-15), 129.3 (C-3a), 130.1 (C-6'), 131.0 (C-3b), 131.1 (C-14), 131.2 (C-9), 131.3 (C-5), 131.7 (C-11b), 131.8 (C-5'), 133.4 (C-1'), 133.8 (C-13), 134.1 (C-4), 139.2 (C-7a), 141.2 (C-2), 144.9 (CH=N), 158.9 (C-6), 160.9 (CONH). IR (KBr) cm⁻¹: 3311 (NH), 1701 (CONH),

1677 (C=O). EI-MS, 70 eV, *m/z* (rel. int., %): 450.00 (3, M⁺), 313.04 (55), 270.04 (100).

N'-(4-Fluorobenzylidene)-6-oxobenzo[4,5]canthine-2-carbohydrazide (**11d**): Yellow solid, yield 70%, mp 274.1–275.8°C; ¹H-NMR (DMSO-*d*₆) δ: 7.34 (t, 2H, H-2', H-6', *J*=8.7 Hz), 7.60 (t, 1H, H-10, *J*=7.8 Hz), 7.80 (t, 1H, H-9, *J*=7.8 Hz), 7.84–7.89 (m, 3H, H-3', H-5', H-14), 8.05 (t, 1H, H-13, *J*=7.8 Hz), 8.47 (d, 1H, H-15, *J*=7.8 Hz), 8.54 (d, 1H, H-11, *J*=7.8 Hz), 8.59 (d, 1H, H-8, *J*=7.8 Hz), 8.80 (s, 1H, N=CH), 9.02 (s, 1H, H-1), 9.16 (d, 1H, H-12, *J*=7.8 Hz), 12.15 (s, 1H, NH). ¹³C-NMR (DMSO-*d*₆) δ: 115.5 (C-1), 116.0 (C-2', C-6'), 116.5 (C-8), 123.9 (C-11), 124.4 (C-11a), 124.6 (C-12), 125.7 (C-10), 128.6 (C-15), 129.2 (C-3a), 129.4 (C-3', C-5'), 130.8 (C-14), 130.9 (C-3b), 131.0 (C-9), 131.1 (C-3b), 131.5 (C-11b), 133.6 (C-5), 133.7 (C-4), 133.8 (C-13), 139.0 (C-7a), 144.9 (C-2), 148.2 (N=CH), 158.7 (C-6), 160.4 (CONH), 163.2 (C-4'). IR (KBr) cm⁻¹: 1676 (C=O). EI-MS, 70 eV, *m/z* (rel. int., %): 434.01 (3, M⁺), 313.04 (40), 270.01 (100).

N'-(4-Dimethylaminobenzylidene)-6-oxobenzo[4,5]canthine-2-carbohydrazide (**11e**): Yellow solid, yield 56%, mp 227.3–229.3°C; ¹H-NMR (DMSO-*d*₆) δ: 3.01 (s, 6H, 2CH₃N), 6.81 (m, 2H, H-3', H-5', *J*=8.7 Hz), 7.62–7.67 (m, 3H, H-2', H-6', H-10), 7.84 (t, H-9, *J*=7.8 Hz), 7.91 (t, H-14, *J*=7.8 Hz), 8.09 (t, 1H, H-13, *J*=7.8 Hz), 8.53 (d, 1H, H-15, *J*=7.8 Hz), 8.61 (d, 1H, H-11, *J*=7.8 Hz), 8.65 (s, 1H, N=CH), 8.66 (d, 1H, H-8, *J*=7.8 Hz), 9.26 (d, 1H, H-12, *J*=7.5 Hz), 9.07 (s, 1H, H-1), 11.95 (s, 1H, N-H). ¹³C-NMR (DMSO-*d*₆) δ: 39.8 (NCH₃), 104.5 (C-11a), 111.9 (C-3', C-5'), 115.3 (C-1), 116.5 (C-8), 121.6 (C-11b), 124.0 (C-11), 124.6 (C-12), 125.7 (C-10), 128.6 (C-2', C-6'), 128.7 (C-15), 129.2 (C-3a), 130.8 (C-14), 130.9 (C-3b), 131.1 (C-9), 131.5 (C-5), 133.6 (C-1'), 133.7 (C-13), 133.8 (C-4), 139.1 (C-7a), 145.3 (C-2), 150.2 (HC=N), 151.6 (C-4'), 158.8 (C-6), 159.8 (CONH). IR (KBr) cm⁻¹: 3311 (NH), 1676 (C=O). EI-MS, 70 eV, *m/z* (rel. int., %): 459.08 (27, M⁺), 313.04 (20), 270.03 (100).

General Procedure for the Synthesis of *N*-Alkyl-6-oxobenzo[4,5]canthine-2-carboxamides (13a–g**)** A solution of methyl 6-oxobenzo[4,5]canthine-2-carboxylate (**9**) (0.3 mmol) in 7.5 mL of a mixture of AcOH and HCl (2:1) was refluxed for 6 h, followed by addition of water (15 mL). The mixture was kept at 0–5°C for 2 h and the precipitate was separated by vacuum filtration and washed several times with water. The 6-oxobenzo[4,5]canthine-2-carboxylic acid (**12**) was obtained in 71% yield.

A solution of compound **12** (0.4 mmol) in SOCl₂ (5 mL) was refluxed for 3 h. The excess of SOCl₂ was removed under vacuum, the residue dissolved in tetrahydrofuran (THF) (15 mL) and the solution cooled to 0°C. To this solution were added triethylamine (1.2 mmol, 3 eq) and the respective amine (0.8 mmol, 2 eq). The reaction mixture was stirred at room temperature for 17–48 h, the solvent removed and the residue dissolved in CHCl₃ (20 mL). The organic layer was washed with 0.5 M HCl (20 mL), 2% aq. Na₂CO₃ (20 mL) and water (3×20 mL), dried over Na₂SO₄ and concentrated under vacuum. Products **13a** and **13c–g** were recrystallized from MeOH, while the product **13f** was recrystallized from water. Product **13b** was purified by chromatographic column (silica gel, CH₂Cl₂, CH₂Cl₂/AcOEt 10 to 50%, AcOEt).

N-Isopropyl-6-oxobenzo[4,5]canthine-2-carboxamide (**13a**): Yellow solid, yield 36%, mp 214.6–217.3°C; ¹H-NMR (CDCl₃) δ: 1.35 (d, 6H, (CH₃)₂CH-NH, *J*=6.6 Hz), 4.26–4.42 (m, 1H,

(CH₃)₂CH–NH), 7.48 (td, 1H, H-10, *J*=7.5, 0.9 Hz), 7.64–7.72 (m, 2H, H-9, H-14), 7.87 (td, 1H, H-13, *J*=7.5, 0.9 Hz), 8.02 (d, 1H, (CH₃)₂CH–NH, *J*=8.1 Hz), 8.09 (d, 1H, H-11, *J*=7.5 Hz), 8.54 (dd, 1H, H-15, *J*=7.5, 0.9 Hz), 8.68 (td, 1H, H-8, H-12, *J*=7.5, 0.9 Hz), 8.81 (s, 1H, H-1). ¹³C-NMR (CDCl₃) δ: 23.2 ((CH₃)₂CH–NH), 41.9 ((CH₃)₂CH–NH), 114.8 (C-1), 117.6 (C-8), 123.1 (C-11), 123.5 (C-12), 125.9 (C-10), 125.1 (C-11a), 129.6 (C-15), 129.8 (C-3a), 130.6 (C-14), 131.2 (C-9), 131.6 (C-5), 131.8 (C-3a), 133.7 (C-13), 134.0 (C-11b), 134.1 (C-4), 139.9 (C-7a), 146.2 (C-2), 159.5 (C-6), 163.7 (CONH). IR (KBr) cm⁻¹: 3390 (NH), 1687 (C=O), 1664 (C=O). EI-MS, 70 eV, *m/z* (rel. int., %): 369.05 (15, M⁺), 270.00 (100), 296.98 (28), 240.99 (29).

N-Butyl-6-oxobenzo[4,5]canthine-2-carboxamide (**13b**): Yellow solid, yield 27%, mp 264.5–266.7°C; ¹H-NMR (CDCl₃) δ: 1.05 (t, 3H, CH₃(CH₂)₃NH, *J*=7.2 Hz), 1.54 (sext, 2H, CH₃CH₂(CH₂)₂NH, *J*=7.2 Hz), 1.76 (quint, 2H, CH₃CH₂CH₂CH₂NH, *J*=7.2 Hz), 3.62 (q, 2H, CH₃(CH₂)₂CH₂NH, *J*=7.2 Hz), 7.56 (t, 1H, H-10, *J*=7.8 Hz), 7.74 (t, 1H, H-14, *J*=7.8 Hz), 7.76 (t, 1H, H-9, *J*=7.8 Hz), 7.92 (t, 1H, H-13, *J*=7.8 Hz), 8.17 (d, 1H, H-11, *J*=7.8 Hz), 8.62 (d, 1H, H-15, *J*=7.8 Hz), 8.75 (d, 2H, H-8, H-12, *J*=7.8 Hz), 8.81 (s, 1H, H-1). ¹³C-NMR (CDCl₃) δ: 14.1 (CH₃(CH₂)₃NH), 20.5 (CH₃CH₂(CH₂)₂NH), 32.2 (CH₃CH₂CH₂CH₂NH), 39.7 (CH₃(CH₂)₂CH₂NH), 114.8 (C-1), 117.6 (C-8), 123.1 (C-11), 123.5 (C-12), 125.0 (C-11a), 125.9 (C-10), 129.6 (C-15), 129.7 (C-3a), 130.6 (C-9), 131.2 (C-14), 131.7 (C-5), 131.8 (C-3b), 133.7 (C-13), 134.0 (C-11b), 134.1 (C-4), 139.8 (C-7a), 146.2 (C-2), 159.4 (C-6), 164.6 (CONH). IR (KBr) cm⁻¹: 3299 (NH), 1685 (C=O). EI-MS, 70 eV, *m/z* (rel. int., %): 355.00 (30, M⁺), 269.98 (100), 296.93 (35), 240.97 (45), 57.97 (85).

N-Pyrrolidyl-6-oxobenzo[4,5]canthine-2-carboxamide (**13c**): Brown solid, yield 56%, mp 234.5–236.7°C; ¹H-NMR (CDCl₃) δ: 2.04 (m, 4H, CH₂CH₂(CH₂)N), 3.84 (m, 2H, CONCH₂), 4.14 (m, 2H, CONCH₂), 7.57 (t, 1H, H-10, *J*=7.5 Hz), 7.76 (t, 1H, H-14, *J*=7.5 Hz), 7.78 (t, 1H, H-9, *J*=7.5 Hz), 7.92 (t, 1H, H-13, *J*=7.8 Hz), 8.18 (d, 1H, H-11, *J*=7.5 Hz), 8.66 (d, 1H, H-15, *J*=7.5 Hz), 8.70 (s, 1H, H-1), 8.77 (d, 1H, H-8, *J*=7.5 Hz), 8.79 (d, 1H, H-12, *J*=7.5 Hz). ¹³C-NMR (CDCl₃) δ: 24.2 (CH₂CH₂(CH₂)N), 27.2 (CH₂CH₂(CH₂)N), 47.8 (CONCH₂), 50.2 (CONCH₂), 117.0 (C-1), 117.7 (C-8), 123.0 (C-11), 125.2 (C-11a), 125.9 (C-10), 129.8 (C-3a), 130.4 (C-9), 131.0 (C-3b), 131.1 (C-14), 131.4 (C-5), 133.7 (C-4), 133.8 (C-13), 134.7 (C-11b), 139.8 (C-7a), 149.8 (C-2), 159.6 (C-6), 166.3 (CON). IR (KBr) cm⁻¹: 1670 (C=O). EI-MS, 70 eV, *m/z* (rel. int., %): 367.03 (30, M⁺), 270.00 (100), 240.97 (40), 69.96 (45).

N-Cyclohexyl-6-oxobenzo[4,5]canthine-2-carboxamide (**13d**): Yellow solid, yield 46%, mp >210°C, decomp; ¹H-NMR (CDCl₃/CD₃OD) δ: 1.40–1.66 (m, 6H, (CH₂(CH₂CH₂)₂CHNH), 1.78–2.08 (m, 4H, (CH₂(CH₂CH₂)₂CHNH), 3.98–4.02 (m, 1H, (CH₂(CH₂CH₂)₂CHNH), 7.52 (t, 1H, H-10, *J*=7.8 Hz), 7.70 (t, 1H, H-14, *J*=7.8 Hz), 7.74 (t, 1H, H-9, *J*=7.8 Hz), 7.81 (t, 1H, H-13, *J*=7.8 Hz), 8.16 (d, 1H, H-11, *J*=7.8 Hz), 8.24 (d, 1H, N–H, *J*=8.7 Hz), 8.57 (d, 1H, H-15, *J*=7.8 Hz), 8.68 (d, 1H, H-8, *J*=7.8 Hz), 8.73 (d, 1H, H-12, *J*=7.8 Hz), 8.82 (s, 1H, H-1). ¹³C-NMR (CDCl₃/CD₃OD) δ: 24.9 (CH₂(CH₂CH₂)₂CHNH), 25.6 (CH₂(CH₂CH₂)₂CHNH), 33.1 (CH₂(CH₂CH₂)₂CHNH), 48.8 (CH₂(CH₂CH₂)₂CHNH), 114.6 (C-1), 117.4 (C-8), 123.0 (C-11), 123.4 (C-12), 125.0 (C-11a), 125.9 (C-10), 129.4 (C-15), 129.5 (C-3a), 130.7 (C-14), 131.2 (C-9), 131.7 (C-3b), 131.8 (C-5), 133.8 (C-6), 134.0 (C-11b), 134.1 (C-4), 139.7 (C-7a),

145.6 (C-2), 159.6 (C-6), 163.9 (CONH). IR (KBr) cm⁻¹: 1679 (C=O). EI-MS, 70 eV, *m/z* (rel. int., %): 395.03 (25, M⁺), 268.88 (100), 312.90 (30), 240.98 (45), 97.89 (95).

N-Benzyl-6-oxobenzo[4,5]canthine-2-carboxamide (**13e**): Yellow solid, yield 44%, mp 157.2–159.0°C; ¹H-NMR (CDCl₃) δ: 4.84 (d, 2H, CH₂NH, *J*=6.5 Hz), 7.34–7.51 (m, 5H, H-2'–H-6'), 7.57 (t, 1H, H-10, *J*=7.5 Hz), 7.75 (td, 2H, H-9, H-14, *J*=7.5, 0.9 Hz), 7.87 (td, 1H, H-13, *J*=7.5, 0.9 Hz), 8.18 (d, 1H, H-11, *J*=7.5 Hz), 8.60 (d, 1H, H-15, *J*=7.5 Hz), 8.60 (t, 1H, N–H, *J*=6.5 Hz), 8.70 (d, 1H, H-12, *J*=7.5 Hz), 8.75 (d, 1H, H-8, *J*=7.5 Hz), 8.93 (s, 1H, H-1). ¹³C-NMR (CDCl₃) δ: 43.9 (CH₂NH), 115.0 (C-1), 117.6 (C-8), 120.0 (C-3', C-5'), 123.1 (C-11), 123.6 (C-12), 125.0 (C-11a), 125.9 (C-10), 127.8 (C-2', C-6'), 129.0 (C-4'), 129.6 (C-15), 129.7 (C-3a), 130.7 (C-14), 131.3 (C-9), 131.8 (C-3b), 131.9 (C-4), 133.8 (C-13), 134.0 (C-11b), 134.2 (C-4), 138.7 (C-5), 139.8 (C-7a), 145.8 (C-8), 159.5 (C-6), 164.7 (CONH). IR (KBr) cm⁻¹: 3406 (NH), 1685 (CONH), 1666 (C=O). EI-MS, 70 eV, *m/z* (rel. int., %): 403.00 (30, M⁺), 269.99 (100), 241.00 (25), 105.93 (70).

N-Methylpiperazyl-6-oxobenzo[4,5]canthine-2-carboxamide (**13f**): Yellow solid, yield 61%, mp 197.2–198.6°C; ¹H-NMR (CDCl₃) δ: 2.35 (s, 3H, CH₃N), 2.52–2.59 (m, 4H, CH₂NCH₃), 3.80–3.92 (m, 4H, CONCH₂), 7.55 (t, 1H, H-10, *J*=7.8 Hz), 7.70 (t, 1H, H-14, *J*=7.8 Hz), 7.72 (t, 1H, C-9, *J*=7.8 Hz), 7.86 (t, 1H, H-13, *J*=7.8 Hz), 8.09 (d, 1H, H-11, *J*=7.8 Hz), 8.46 (s, 1H, H-1), 8.60 (d, 1H, H-15, *J*=7.8 Hz), 8.70 (d, 1H, H-12, *J*=7.8 Hz), 8.74 (d, 1H, H-8, *J*=7.8 Hz). ¹³C-NMR (CDCl₃) δ: 42.3 (CH₃N), 45.7 (CH₂NCH₃), 47.1 (CH₂NCH₃), 54.7 (CONCH₂), 55.4 (CONCH₂), 117.10 (C-1), 117.7 (C-8), 123.0 (C-11), 123.6 (C-12), 124.9 (C-11a), 125.9 (C-10), 129.6 (C-15), 129.8 (C-3a), 130.6 (C-14), 130.8 (C-11b), 131.6 (C-3b), 131.3 (C-9), 133.9 (C-13), 134.0 (C-4), 134.5 (C-5), 139.8 (C-7a), 148.9 (C-2), 159.6 (C-6), 167.7 (CONH). IR (KBr) cm⁻¹: 3450 (NH), 1685 (C=O). EI-MS, 70 eV, *m/z* (rel. int., %): 396.03 (5, M⁺), 268.89 (100), 338.99 (85), 296.89 (70), 240.97 (40).

N-Morpholyl-6-oxobenzo[4,5]canthine-2-carboxamide (**13g**): Yellow solid, yield 30%, mp 242.3–243.0°C; ¹H-NMR (CDCl₃) δ: 3.86–3.95 (m, 8H, N(CH₂CH₂)₂O), 7.57 (dd, 1H, H-10, *J*=7.8, 1.2 Hz), 7.73–7.81 (m, 2H, H-9, H-14), 7.92 (dd, 1H, H-13, *J*=7.8, 1.2 Hz), 8.15 (ddd, 1H, H-11, *J*=7.8, 1.2, 0.9 Hz), 8.65 (ddd, 1H, H-15, *J*=7.8, 1.2, 0.9 Hz), 8.74 (ddd, 1H, H-18, *J*=7.8, 1.2, 0.9 Hz), 8.79 (dt, 1H, H-15, *J*=7.8, 1.2 Hz), 8.47 (s, 1H, H-1). ¹³C-NMR (CDCl₃) δ: 48.6 (NCH₂CH₂O), 67.5 (NCH₂CH₂O), 117.1 (C-1), 117.7 (C-8), 123.0 (C-11), 123.6 (C-12), 125.0 (C-11a), 125.9 (C-10), 129.6 (C-15), 129.8 (C-3a), 130.6 (C-14), 131.3 (C-9), 131.6 (C-5, C-3b), 133.9 (C-13), 134.0 (C-11b), 134.5 (C-4), 139.8 (C-7a), 149.0 (C-2), 159.6 (C-6), 167.6 (CON). IR (KBr) cm⁻¹: 1679 (C=O). EI-MS, 70 eV, *m/z* (rel. int., %): 383.01 (25, M⁺), 269.99 (100), 240.98 (25), 85.99 (25).

In Vitro Antitumor Assays The synthesized compounds were evaluated *in vitro* against seven human tumor cell lines consisting of glioma (U251), breast (MCF-7), resistant ovarian (NCI/ADR-RES), renal (786-0), lung (NCI-H460), ovarian (OVCAR-03) and colon (HT-29), according Monks *et al.* protocol.²³ Cell lines were obtained from National Cancer Institute at Frederick, MA, U.S.A. Stock cultures were grown in medium containing 5 mL RPMI 1640 (GIBCO BRL) supplemented with 5% fetal bovine serum (FBS, GIBCO), at 37°C with 5% CO₂. Penicillin–streptomycin (1000 μg/L: 1000 U/L, 1 mL/L) was added to the experimental cultures.

Cells in 96-well plates ($100\mu\text{L}$ cells well^{-1}) were exposed to compounds **11a–e** and **13a–g** in dimethyl sulfoxide (DMSO) (concentrations of 0.25, 2.5, 25, $250\mu\text{g mL}^{-1}$) at 37°C , 5% of CO_2 in air for 48 h. Final DMSO concentration did not affect the cell viability. Afterwards cells were fixed with 50% trichloroacetic acid and cell proliferation determined by spectrophotometric quantification (540 nm) of cellular protein content by using sulforhodamine B assay and doxorubicin as the positive control. Using the concentration–response curve for each cell line, the IC_{50} value (drug concentration that produces a 50% reduction in cellular growth when compared to untreated control cells) were determined through nonlinear regression analysis using software ORIGIN 8.0® (OriginLab Corporation). Compounds with IC_{50} values $\geq 100\mu\text{M}$ were considered not active.

Parasites and Cells Epimastigote form of *Trypanosoma cruzi* Y strain were maintained at 28°C by weekly transfers in liver infusion tryptose medium (LIT) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco Invitrogen Corporation, New York, U.S.A.). *Leishmania amazonensis* MHOM/BR/75/Josefa strain used in the present study was isolated from a patient with diffuse cutaneous leishmaniasis by C.A. Cuba-Cuba (Universidade de Brasília, Brazil). Promastigotes were cultured at 25°C in Warren's medium (brain heart infusion plus haemin and folic acid) pH 7.0, supplemented with 10% FBS in a tissue flask with weekly transfers. J774G8 murine macrophages were maintained in tissue flasks with RPMI 1640 medium (Gibco Invitrogen Corporation, New York, U.S.A.) pH 7.6, with sodium bicarbonate and L-glutamine added, and supplemented with 10% FBS at 37°C in a 5% CO_2 –air mixture.

Antiprotozoal Assay The effects of synthesized compounds **11a–e** and **13a–g** were evaluated in promastigote forms of *L. amazonensis* and epimastigote forms of *T. cruzi* Y strain. The inocula consisted of protozoa in logarithmic growth phase (1×10^6 cells/mL) was introduced into 24-well plate containing the compounds dissolved in DMSO and LIT medium in several concentrations (2 to $300\mu\text{M}$). The final concentration of DMSO did not exceed 1%. Cell grown was determined by counting the parasites with a Neubauer hemocytometer after incubation for 72 h at 25°C for *L. amazonensis* and 96 h at 28°C for *T. cruzi*. The results were expressed as percentage of inhibition in relation to the control cultured. The 50% inhibitory concentration (IC_{50}) was determined by logarithm regression analysis of the data obtained.

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