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Article

Microwave-Assisted Synthesis and Antileishmanial Activity of 3-methoxycarbonyl-γ-butyrolactone Derivatives

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Descrevemos a síntese assistida por micro-ondas de dez derivados de 3-metoxicarbonil-γ-butirolactona e a avaliação *in vitro* da atividade leishmanicida desses compostos contra formas promastigotas de *Leishmania amazonensis*. A síntese forneceu a maioria dos compostos com 80-95% de rendimento e as reações duraram cerca de 10-20 min. A maior parte dos compostos apresentaram valores de IC_{50} superiores a 400 uM. Os compostos **5** (*trans*-3-(*p*-metoxi)benzil-4-metil-3-metoxicarbonil-γ-butirolactona) e **10** (*trans*-3-(*p*-metoxi)benzil-4-benzil-3-metoxicarbonil-γ-butirolactona) foram as exceções, pois exibiram valores de IC_{50} de 170,4 e 179,6 μM, respectivamente, o que sugere que a atividade leishmanicida das 3-metoxicarbonil-γ-butirolactonas pode estar relacionada com a natureza e o tamanho do substituinte na posição C-4.

We describe the microwave-assisted synthesis of ten 3-methoxycarbonyl-γ-butyrolactone derivatives and evaluate their *in vitro* antileishmanial activity against promastigote forms of *Leishmania amazonensis*. The synthesis furnished most of the compounds in 80-95% yield and reactions lasted about 10-20 min. Most of the compounds displayed IC_{50} values higher than 400 μM. Compounds **5** (*trans*-3-(*p*-methoxy)benzyl-4-methyl-3-methoxycarbonyl-γ-butyrolactone) and **10** (*trans*-3-(*p*-methoxy)benzyl-4-benzyl-3-methoxycarbonyl-γ-butyrolactone) were the exceptions: they displayed IC_{50} values of 170.4 and 179.6 μM, respectively, suggesting that the leishmanicidal activity of 3-methoxycarbonyl-γ-butyrolactones may be related to the nature and size of the substituent at position C-4.

Keywords: γ-butyrolactone, multicomponent reaction, microwave-assisted synthesis, *Leishmania amazonensis*

Introduction

Protozoan parasites of the *Leishmania* genus cause a group of diseases known as Leishmaniasis, a parasitosis that affects more than 12 million people worldwide and accounts for high mortality rates in tropical and subtropical countries.[1,2] It is estimated that two to three million new leishmaniasis cases emerge each year, and that some 350 million people are at risk of infection.[3] The parasites of the genus *Leishmania* can infect humans and several species of mammals. The infection manifests itself in three main typical ways: visceral, cutaneous, and mucocutaneous leishmaniasis.[4]

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Pentavalent antimonials were first used in the clinical setting at the beginning of the last century; they remain the first-choice drugs to treat leishmaniasis. However, these compounds are toxic and poorly tolerated, require daily injections for up to 28 days, and are becoming ineffective due to proliferation of resistant parasites.[5] Second-line drugs, like amphotericin B and pentamidine, are options in combined therapy or in cases of antimony treatment failure.[6,7] Therefore, the development of new antileishmanial compounds is imperative.[1]

γ-Butyrolactones are a structural feature of a diversity of natural products, such as sesquiterpene lactones[8-10] and lignan lactones.[11,12] The γ-lactone subunit has been associated with a number of anti-parasitic activities; e.g., leishmanicidal,[13,14] nematocidal,[15] and antiplasmodial

actions,[16] among several other biological activities.[17-19] In this scenario, the interest in developing new methodologies to synthesize compounds bearing γ-butyrolactones in their structures has increased, with emphasis on multicomponent reactions (MCRs).[20-24] MCRs are advantageous: they incorporate most, if not all atoms of the reagents in the final product in few steps, usually involve a one-pot reaction, and allow for facile product purification.[25] A few synthetic γ-butyrolactones have been evaluated for their antileishmanial activity.

Microwave irradiation has gained popularity in the past decade as a powerful tool to rapidly and efficiently synthesize a variety of compounds. This process is advantageous over conventional thermal heating: it reduces reaction times, improves yields, and suppresses the generation of side products.[26-28]

As part of our ongoing project on the synthesis and biological activities of γ-butyrolactones,[29-32] in this paper we report the use of microwave irradiation as heat source to promote the fast and efficient synthesis of a series of 3,3,4-trisubstituted and 3,3,4,4-tetrasubstituted 3-methoxycarbonyl-γ-butyrolactone derivatives obtained by the multicomponent reaction previously developed by Le Gall and co-workers.[33] Here, we have also evaluated the antileishmanial activity of these compounds.

Results and Discussion

We obtained the target compounds by MCR (see Scheme 1), preferably using microwave irradiation as heat source. Only compound **9** could not be achieved by microwave irradiation, but was obtained in good yield after 3 h under conventional heating. Table 1 summarizes the results of these reactions.

Figure 1 shows the chemical structures of the 3-methoxycarbonyl-γ-butyrolactones **1**-**10**. Compounds **1**-**4** have been synthesized previously,[33] but compounds **5**-**10** are new.

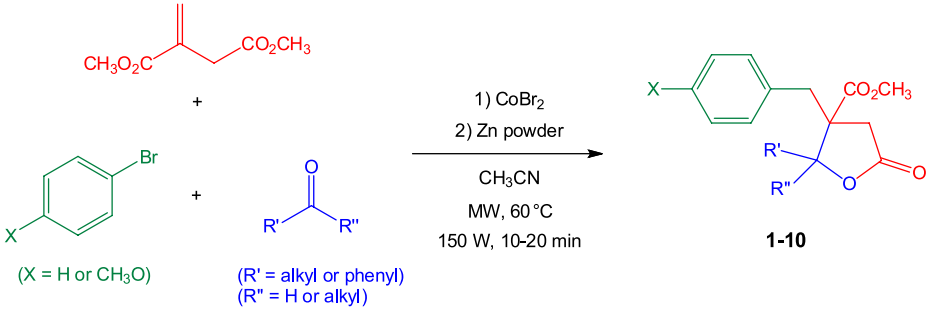
We identified the structures of the synthesized compounds on the basis of ^{1}H and ^{13}C NMR data, as well as two-dimensional NMR techniques (HMQC, HMBC and ^{1}H-^{1}H COSY). We assigned the relative stereochemistry of these compounds on the basis of NOE difference spectroscopy, by irradiating the signal of the hydrogen at C-4 (for compounds **1**, **2**, **5**, **6**, and **10**) or the methyl group at C-4 (for compounds **3**, **8**, and **9**) with respect to the hydrogen at C-2.

In the biological assays, we tested all the compounds as mixtures of enantiomers. Table 2 lists the results of the *in vitro* evaluation of the leishmanicidal activity of the 3-methoxycarbonyl-γ-butyrolactones **1**-**10** against promastigote forms of *Leishmania amazonensis* (MHOM/BR/PH8). Most of the tested compounds did not cause significant lysis at concentrations lower than 50 μM (data not shown). The most active compounds at 50 μM

Table 1. Yields obtained in the synthesis of compounds **1**-**10** produced via Scheme 1[a]

Compound	Conventional heating at 60 °C (oil bath)		Microwave irradiation at 60 °C and 150 W	
	Reaction time / min	Yield / %[b]	Reaction time / min	Yield / %[b]
1	180	35	20	85
2	60	70	10	96
3	60	13	20	82
4	180	20	20	80
5	120	18	10	81
6	180	24	10	88
7	180	13	10	86
8	60	30	10	85
9	180	78	10-30	0
10	60	83	20	90

[a]The reactions were conducted under argon atmosphere with 5 mL of acetonitrile, zinc powder (0.8 g, 12 mmol), dimethyl itaconate (2 g, 13 mmol), an aldehyde or a ketone (2.5 mmol), and the aryl bromide (4 mmol). After brief stirring at room temperature, cobalt bromide (0.13 g, 0.6 mmol), trifluoroacetic acid (0.03 mL), and 1,2-dibromoethane (0.05 mL) were successively added. The reaction mixture was heated at 60 °C for 1-3 h in an oil bath, or irradiated for 10-20 min in a CEM Discovery® focused microwave oven at 60 °C and 150 W; [b]isolated yield.



Scheme 1. Preparation of compounds **1**-**10** by the multicomponent reaction developed by Le Gall and co-workers.[33]

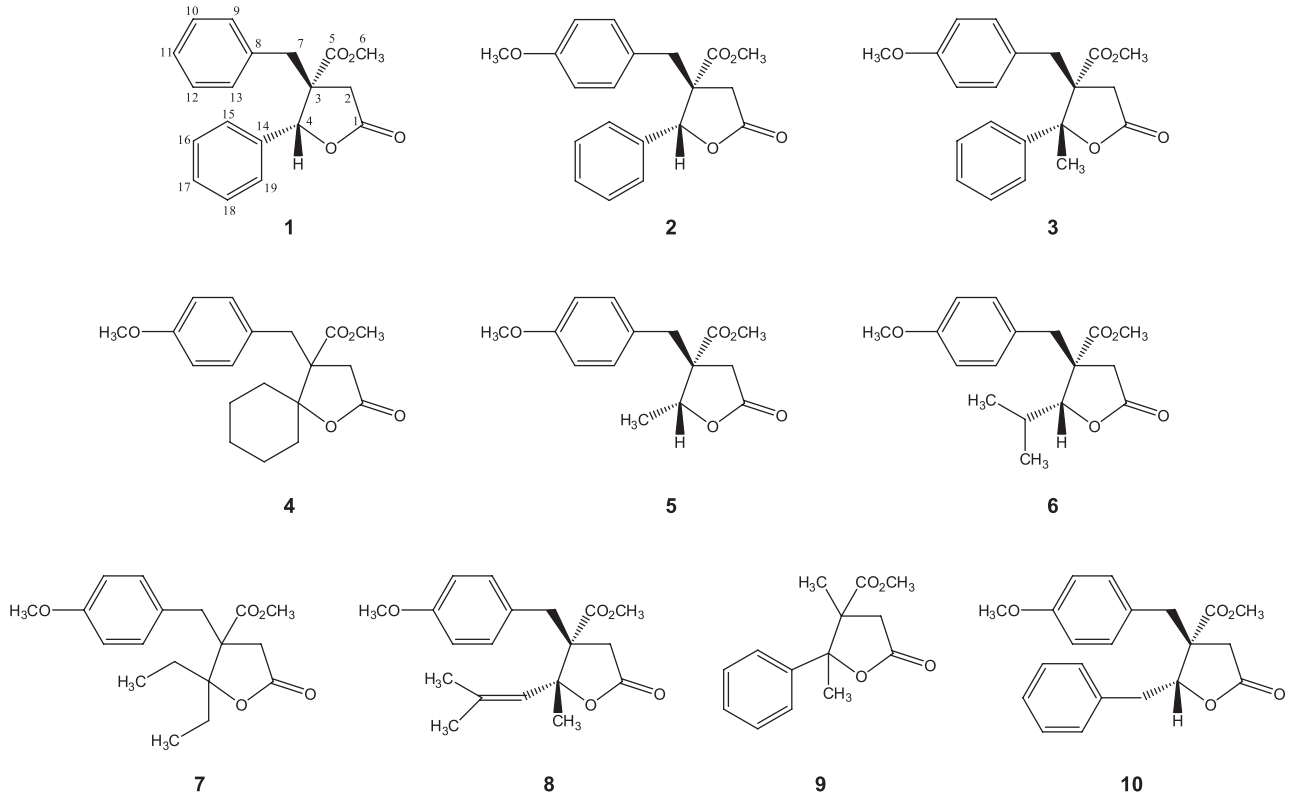


Figure 1. Chemical structures of the 3-methoxycarboxyl-γ-butyrolactone derivatives **1-10**.

Table 2. *In vitro* leishmanicidal activity of the 3-methoxycarbonyl-γ-butyrolactone derivatives **1-10** [3,34]

Compound	% Lysis ± SD/concentration / μM[a]				IC_{50}
	50	100	200	400	
1	5.6 ± 5.1	29.6 ± 2.7	40.1 ± 4.9	42.3 ± 5.7	412.2
2	11.4 ± 8.2	28.5 ± 6.1	35.6 ± 7.2	46.0 ± 4.3	414.9
3	14.2 ± 5.8	25.0 ± 7.3	29.1 ± 1.8	30.0 ± 0.7	467.4
4	4.9 ± 1.9	9.6 ± 4.4	43.5 ± 6.4	49.5 ± 2.4	401.9
5	15.8 ± 4.6	38.1 ± 8.9	57.7 ± 4.7	68.8 ± 4.8	170.4
6	0	0	22.1 ± 4.5	40.8 ± 7.7	415.6
7	0	0	20.61 ± 4.4	21.9 ± 4.1	1045
8	0	38.4 ± 2.8	35.0 ± 0.7	44.0 ± 3.1	405.1
9	0	1.8 ± 3.1	3.0 ± 5.2	8.8 ± 8.6	1989
10	6.2 ± 2.1	22.2 ± 4.3	54.2 ± 1.5	81.3 ± 0.8	179.6

[a]Percentages of lysis at concentrations of 6.25, 12.5, and 25 μM were not statistically significant for compounds **1-3**. No lyses were observed at these concentrations for compounds **4-10**. Positive control: Amphotericin B (12 μM): IC_{50} = 13.7 μM.

were **2** (11.4 ± 8.2% lysis), **3** (14.2 ± 5.8% lysis), and **5** (15.8 ± 4.6% lysis). On the other hand, γ-butyrolactone **8** was found to be the most active among the tested compounds at 100 μM, causing 38.4 ± 2.8% lysis. These data indicated that compound **8** did not display a dose-dependent effect at concentrations lower than 100 μM. The γ-butyrolactones **5** (68.8 ± 4.8%) and **10** (81.3 ± 0.8%) afforded the highest lysis value at 400 μM; their IC_{50} (Inhibitory Concentration) values were 170.4 μM and 179.6 μM, respectively, much higher than that of amphothericin B (IC_{50} = 13.7 μM), used as positive control.

When we related the IC_{50} values to the structures of the γ-butyrolactone derivatives, we noticed that the antileishmanial activity decreased when the aromatic ring did not bind to the β-methyl group, as in the case of compound **9**. The methoxyl group at the aromatic moiety did not seem to increase the leishmanicidal action, as evidenced by the comparison between the IC_{50} values of compounds **1** and **2**. In addition, comparison among the IC_{50} values of compounds **2-8** and **10** clearly indicated that the leishmanicidal activity of these compounds depended on the nature of the substituent at C-4. Although these results demonstrated that the methyl and benzyl groups at position γ play a key role in the leishmanicidal activity of compounds **5** and **10**, further studies are necessary to investigate the mechanism through which these substituents affect the leishmanicidal activity of this class of compounds.

Conclusion

In summary, our results indicated that the microwave-assisted synthesis significantly increased the product yields

and reduced reaction time as compared with the conventionally heated systems. Moreover, the antileishmanial activity of the 3-methoxycarbonyl-γ-butyrolactone derivatives was not so strong as compared with that of amphotericin B and other antileishmanial drugs currently employed in the clinical setting. However, compounds **5** and **10**, which proved to be the most active among the evaluated compounds, should be further investigated, mainly because their synthesis by microwave-assisted synthesis reduced reaction times and improved yields.

Experimental

General

Mass spectra were acquired on an UltroTOF-Q mass spectrometer (Bruker Daltonics, Billerica, MA, USA) fitted with an ESI operating in the positive ion mode. Accurate masses were obtained using TFA-Na^+ (sodiated trifluoroacetic acid) as the internal standard. ^{1}H-NMR spectroscopy was performed using a Bruker DPRX-400 instrument (Bruker, Fällanden, Switzerland) operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C. TMS was used as internal standard. The chemical shifts are reported in ppm (δ); coupling constants (J) values are given in Hertz (Hz). Signal multiplicities are represented by: s (singlet), d (doublet), dd (double doublet), dq (double quadruplet), m (multiplet). Unless noted otherwise, all the solvents and reagents were commercially available and used without further purification.

General procedure for the synthesis of compounds **1**-**10**

The 3-methoxycarbonyl-γ-butyrolactones **1**-**10** were synthesized using the one-pot multicomponent methodology described by Le Floch and co-workers.[33] All the reactions were performed in duplicate: one employed a conventional thermal heating with an oil bath; the other used microwave radiation as heat source. A 25-mL round-bottomed flask under argon atmosphere was charged with previously purified[34] acetonitrile (5 mL), zinc dust (0.8 g, 12 mmol), dimethyl itaconate (2 g, 13 mmol), an aldehyde or a ketone (2.5 mmol), and the aryl bromide (4 mmol). This mixture was briefly stirred at room temperature. Cobalt bromide (0.13 g, 0.6 mmol), trifluoroacetic acid (0.03 mL), and 1,2-dibromoethane (0.05 mL) were then added successively to the previous mixture. The reaction mixture was heated at 60 °C for 1-3 h in an oil bath, or irradiated for 10-20 min in a CEM Discovery® focused microwave oven at 60 °C and 150 W. The reaction mixture was monitored by thin-layer chromatography until consumption of the aryl bromide was complete (between 1 and 3 h for conventional thermal heating, and between 10 and 20 min for microwave irradiation heating). Next, the reaction mixture was then filtered through Celite®, which was then washed several times with anhydrous diethyl ether. The organic fractions were combined and concentrated under reduced pressure. The crude reaction product was purified by flash column chromatography through silica gel (*n*-hexane/ethyl acetate, 7:3 v/v), to afford a mixture of stereoisomers of the butyrolactone with yields ranging from 13 to 83% for conventional thermal heating, and from 80 to 96% for microwave-assisted heating, with the exception of compound **9**, which was not obtained by microwave irradiation.

trans-3-Benzyl-4-phenyl-3-methoxycarbonyl-γ-butyrolactone (**1**)

Yields: 35% (conventional thermal heating), 85% (microwave radiation); IR (KBr) ν/cm^{-1} 3460, 1786, 1736, 1216; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.45 (m, 2H, 2Ar-H), 7.43 (m, 2H, 2Ar-H), 7.35 (m, 2H, 2Ar-H), 7.23 (m, 2H, 2Ar-H), 6.95 (m, 2H, 2Ar-H), 5.67 (s, 1H, CH), 3.75 (s, 3H, CH_3), 3.12 (d, 1H, J 17.6 Hz, CH_2), 2.86 (d, 1H, J 13.9 Hz, CH_2), 2.71 (d, 1H, J 17.6, Hz CH_2), 2.17 (d, 1H, J 13.9 Hz, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 74.5, 173.0, 135.7, 134.3, 129.5, 129.1, 128.5, 127.3, 126.5, 85.7, 56.3, 52.9, 39.0, 35.6; ESI-MS *m/z* calcd. for $C_{19}H_{18}NaO_4^+$: 333.1103, found: 333.1097 $[M+Na]^+$.

trans-3-(*p*-Methoxy)benzyl-4-phenyl-3-methoxycarbonyl-γ-butyrolactone (**2**)

Yields: 70% (conventional thermal heating), 96% (microwave radiation); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.43 (m, 2H, 2Ar-H), 7.42 (m, 1H, Ar-H), 7.35 (m, 2H, 2Ar-H), 7.23 (m, 2H, 2Ar-H), 6.85 (d, 2H, J 8.1 Hz, 2Ar-H), 6.74 (d, 2H, J 8.1 Hz, 2Ar-H), 5.73 (s, 1H, CH), 3.76 (s, 3H, OCH_3), 3.73 (s, 3H, CH_3), 3.12 (d, 1H, J 17.7 Hz, CH_2), 2.80 (d, 1H, J 13.8 Hz, CH_2), 2.69 (d, 1H, J 17.7 Hz, CH_2), 2.12 (d, 1H, J 13.8 Hz, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 174.6, 173.1, 158.8, 134.4, 130.6, 129.1, 128.6, 127.6, 126.6, 114.0, 86.0, 56.4, 55.6, 52.8, 38.5, 35.7; ESI-MS *m/z* calcd. for $C_{20}H_{20}NaO_5^+$: 363.1208, found: 363.1201 $[M+Na]^+$.

trans-3-(*p*-Methoxy)benzyl-4-methyl-4-phenyl-3-methoxycarbonyl-γ-butyrolactone (**3**)

Yields: 13% (conventional thermal heating), 82% (microwave radiation); IR (KBr) ν/cm^{-1} 3450, 2980, 1782, 1768, 1734, 1730, 1512, 1250; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.34 (m, 2H, 2Ar-H), 7.33 (m, 1H, Ar-H), 7.30 (m, 2H, 2Ar-H), 7.00 (d, 2H, J 8.6 Hz, 2Ar-H), 6.80 (d, 2H, J 8.6 Hz, 2Ar-H), 3.77 (s, 3H, OCH_3), 3.65 (d, 1H, J 13.4 Hz, CH_2), 3.31 (s, 3H, CH_3), 2.91 (d, 1H, J 17.4 Hz, CH_2), 2.77 (d, 1H, J 13.4 Hz, CH_2), 2.62 (d, 1H, J 17.4 Hz, CH_2), 1.95 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 174.6, 170.9,

158.8, 140.5, 131.0, 128.4, 128.3, 127.6, 125.0, 114.0, 89.2, 60.1, 55.2, 52.0, 37.0, 35.7, 22.9; ESI-MS *m/z* calcd. for $C_{21}H_{22}NaO_5^+$: 377.1365, found: 377.1367 $[M+Na]^+$.

trans-3-(*p*-Methoxy)benzyl-4,4-cyclohexyl-3-methoxycarbonyl-γ-butyrolactone (**4**)

Yields: 20% (conventional thermal heating), 80% (microwave radiation); IR (KBr) ν/cm^{-1} 3440, 1730, 1704, 1500, 1250; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.88 (d, 2H, *J* 8.8 Hz, 2Ar-H), 6.74 (d, 2H, *J* 8.8 Hz, 2Ar-H), 3.71 (s, 3H, OCH_3), 3.67 (s, 3H, CH_3), 3.32 (d, 1H, *J* 13.4 Hz, CH_2), 3.00 (d, 1H, *J* 17.9 Hz, CH_2), 2.50 (d, 1H, *J* 17.9 Hz, CH_2), 2.48 (d, 1H, *J* 13.4 Hz, CH_2), 2.00 (m, 2H, CH_2), 1.65 (m, 2H, CH_2), 1.56 (m, 2H, CH_2), 1.25 (m, 2H, CH_2), 1.15 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.9, 171.5, 158.4, 130.5, 127.3, 113.8, 87.3, 58.2, 54.9, 52.0, 36.3, 34.9, 33.2, 30.2, 25.0, 22.3, 21.8; ESI-MS *m/z* calcd. for $C_{19}H_{24}NaO_5^+$: 355.1521, found: 355.1523 $[M+Na]^+$.

trans-3-(*p*-Methoxy)benzyl-4-methyl-3-methoxycarbonyl-γ-butyrolactone (**5**)

Yields: 18% (conventional thermal heating), 81% (microwave radiation); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.02 (d, 2H, *J* 8.6 Hz, 2Ar-H), 6.84 (d, 2H, *J* 8.6 Hz, 2Ar-H), 4.54 (q, 1H, *J* 6.5 Hz, CH), 3.80 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 3.35 (d, 1H, *J* 14.0 Hz, CH_2), 2.90 (d, 1H, *J* 17.7 Hz, CH_2), 2.73 (d, 1H, *J* 14.0 Hz, CH_2), 2.55 (d, 1H, *J* 17.7 Hz, CH_2), 1.35 (d, 3H, *J* 6.5 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.5, 171.9, 159.0, 130.9, 127.4, 114.2, 81.8, 56.0, 55.3, 52.5, 40.0, 35.3, 16.6; ESI-MS *m/z* calcd. for $C_{15}H_{18}NaO_5^+$: 301.1052, found: 301.1028 $[M+Na]^+$.

trans-3-(*p*-Methoxy)benzyl-4-isopropyl-3-methoxycarbonyl-γ-butyrolactone (**6**)

Yields: 24% (conventional thermal heating), 88% (microwave radiation); IR (KBr) ν/cm^{-1} 3450, 2940, 1778, 1734, 1500, 1250; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7,00 (d, 2H, *J* 8.7 Hz, 2Ar-H), 6.81 (d, 2H, *J* 8.7 Hz, 2Ar-H), 4.13 (d, 1H, *J* 5.7 Hz, CH), 3.77 (s, 3H, OCH_3), 3.74 (s, 3H, CH_3), 3.43 (d, 1H, *J* 14.0 Hz, CH_2), 2.80 (d, 1H, *J* 17.6 Hz, CH_2), 2.73 (d, 1H, *J* 14.0 Hz, CH_2), 2.56 (d, 1H, *J* 17.6 Hz, CH_2), 1.96 (dq, 1H, *J* 5.7, 6.7 Hz, CH), 1.12 (d, 3H, *J* 6.7 Hz, CH_3), 0.95 (d, 3H, *J* 6.7 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 174.7, 172.2, 158.9, 131.0, 127.4, 114.0, 90.9, 55.2, 55.1, 52.4, 41.8, 36.5, 30.5, 20.3, 17.7; ESI-MS *m/z* calcd. for $C_{17}H_{22}NaO_5^+$: 329.1365, found: 329.1341 $[M+Na]^+$.

3-(*p*-Methoxy)benzyl-4,4-diethyl-3-methoxycarbonyl-γ-butyrolactone (**7**)

Yields: 13% (conventional thermal heating), 86% (microwave radiation); IR (KBr) ν/cm^{-1} 3500, 2950, 1778, 1726, 1510, 1248; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.92 (2H, d, *J* 8.6 Hz, 2Ar-H), 6.74 (2H, d, *J* 8.6 Hz, 2Ar-H), 3.71 (3H, s, CH_3), 3.65 (3H, s, OCH_3), 3.38 (1H, d, *J* 13.2 Hz, CH_2), 2.96 (1H, d, *J* 18.0 Hz, 2Ar-H), 2.57 (1H, d, *J* 13.2 Hz, 2Ar-H), 2.55 (1H, d, *J* 18.0 Hz, CH_2), 1.94 (1H, dq, *J* 11.9 Hz, 7.3, CH_2), 1.92 (1H, dq, *J* 14.9 Hz, 7.5, CH_2), 1.70 (1H, dq, *J* 14.9 Hz, 7.5, CH_2), 1.55 (1H, dq, *J* 11.9 Hz, 7.3, CH_2), 1.04 (3H, d, *J* 7.3 Hz, CH_3), 0.90 (3H, d, *J* 7.5 Hz, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 174.2, 172.0, 158.7, 131.0, 127.7, 114.0, 91.2, 58.7, 55.2, 52.2, 37.3, 36.5, 26.3, 24.8, 8.30, 8.06; ESI-MS *m/z* calcd. for $C_{18}H_{25}O_5^+$: 321.1702, found: 321.1694 $[M+H]^+$.

3-(*p*-Methoxy)benzyl-4-methyl-4-isopentenyl-3-methoxycarbonyl-γ-butyrolactone (**8**)

Yields: 30% (conventional thermal heating), 85% (microwave radiation); IR (KBr) ν/cm^{-1} 3500, 2950, 1782, 1732, 1515, 1252; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.88 (d, 2H, *J* 8.8 Hz, 2Ar-H), 6.73 (d, 2H, *J* 8.8 Hz, 2Ar-H), 5.51 (s, 1H, =CH), 3.70 (s, 3H, OCH_3), 3.68 (s, 3H, CH_3), 3.39 (d, 1H, *J* 14.0 Hz, CH_2), 2.95 (d, 1H, *J* 17.9 Hz, CH_2), 2.46 (d, 1H, *J* 17.9 Hz, CH_2), 2.39 (d, 1H, *J* 14.0 Hz, CH_2), 1.83 (s, 3H, CH_3), 1.77 (s, 3H, CH_3), 1.64 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 174.1, 172.0, 158.7, 136.6, 130.7, 128.0, 121.0, 114.1, 88.2, 59.0, 55.2, 52.4, 38.1, 34.3, 28.0, 24.6, 19.2; ESI-MS *m/z* calcd. for $C_{19}H_{25}O_5^+$: 333.1702, found: 333.1695 $[M+H]^+$.

3,4-Dimethyl-4-phenyl-3-methoxycarbonyl-γ-butyrolactone (**9**)

Yields: 78% (conventional thermal heating), 0% (microwave radiation); IR (KBr) ν/cm^{-1} 2960, 1788, 1732, 1230; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.34 (s, 1H, Ar-H), 7.33 (m, 2H, 2Ar-H), 7.32 (m, 2H, 2Ar-H), 3.27 (s, 3H, CH_3), 3.12 (d, 1H, *J* 17.4 Hz, CH_2), 2.55 (d, 1H, *J* 17.4 Hz, CH_2), 1.78 (s, 3H, CH_3), 1.54 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 174.3, 172.5, 140.3, 128.2, 128.1, 124.5, 89.2, 54.4, 52.1, 39.9, 23.8, 19.2; ESI-MS *m/z* calcd. for $C_{14}H_{16}NaO_4^+$: 271.0946, found: 271.0957 $[M+Na]^+$.

trans-3-(*p*-Methoxy)benzyl-4-benzyl-3-methoxycarbonyl-γ-butyrolactone (**10**)

Yields: 83% (conventional thermal heating), 90% (microwave radiation); IR (KBr) ν/cm^{-1} 3450, 1784, 1722, 1500, 1230; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.32 (m, 2H, 2Ar-H), 7.30 (m, 2H, 2Ar-H), 7.20 (m, 1H, Ar-H), 7.03 (d, 2H, *J* 8.6 Hz, 2Ar-H), 6.86 (d, 2H, *J* 8.6 Hz, 2Ar-H), 4.55 (dd, 1H, *J* 3.1 Hz, CH), 3.77 (s, 3H, OCH_3), 3.76 (s, 3H, CH_3), 3.33 (d, 1H, *J* 13.9 Hz, CH_2), 2.97 (dd, 1H, *J* 11.6, 3.1 Hz, CH_2), 2.82 (d, 1H, *J* 17.5 Hz, CH_2), 2.81 (d, 1H, *J* 13.9 Hz, CH_2), 2.80

(dd, 1H, *J* 11.6, 3.1 Hz, CH_2), 2.57 (d, 1H, *J* 17.5 Hz, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 174.2, 171.9, 158.9, 136.4, 131.0, 129.3, 128.0, 127.2, 127.1, 114.1, 85.9, 55.6, 55.3, 52.5, 39.8, 37.2, 36.2; ESI-MS *m/z* calcd. for $C_{21}H_{22}NaO_5^+$: 377.1365, found: 377.1344 $[M+Na]^+$.

Antileishmanial assays

The bioassays were performed using *Leishmania amazonensis* (MHOM/BR/PH8). Promastigote forms of *L. amazonensis* were incubated in M199 medium (Gibco), supplemented with L-glutamine (2 mM), $NaHCO_3$ (10 mM), penicillin (100 UI mL^{-1}), streptomycin (100 μg mL^{-1}), and 20% bovine fetal serum (Gibco). After six days of the initial inoculation, promastigote forms (2×10^6 parasites mL^{-1}) were incubated in 96-well microtiter plates containing the tested samples. The γ-butyrolactones derivatives were dissolved in dimethyl sulfoxide (DMSO) and diluted into the medium, to give final concentrations of 6.25, 12.5, 25.0, 50.0, 100.0, 200.0, and 400 μM. The plates were incubated at 22 °C for 24 h, and the lysis percentage was determined by an MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide)] (Sigma-Aldrich) colorimetric method.[35] The bioassays were performed in triplicate, using M199 medium with 0.5% DMSO as negative control and amphotericin B (Sigma-Aldrich) as positive control group.

Statistical analysis

The obtained data are represented as mean ± S.D. The data were statistically analyzed by one-way ANOVA using GraphPad Prism 5.0 software, followed by Tukey's multiple comparison test. The IC_{50} (inhibitory concentration necessary to cause lysis of 50% of parasites) values were calculated using sigmoid dose-response curves.

Supplementary Information

^1H NMR, ^{13}C NMR, IR and mass spectra of compounds, and the curves used to calculate the IC_{50} values, are available free of charge at http://jbcs.sbq.org.br as a PDF file.

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