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In vitro and in vivo anti-Leishmania activity of polysubstituted synthetic chalcones

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ABSTRACT

The in vitro screening of 43 polysubstituted chalcones against *Leishmania amazonensis* axenic amastigotes, led to the evaluation of 9 of them in a macrophage-infected model with the two other most infectious *Leishmania* species prevalent in Peru (*L. braziliensis* and *L. peruviana*). The five most active and selective chalcones were studied in vivo, resulting on the identification of two chalcones with high reduction parasite burden percentages.

Leishmaniasis is a vector-borne disease caused by protozoan parasites of the genus Leishmania. It is transmitted through the bite of female phlebotomine sandflies and can range from mild self-healing cutaneous lesions to lethal visceral leishmaniasis. Different clinical manifestations of the various forms of leishmaniasis are given by different types of infected tissues, host genetic factors and immune responses. Leishmaniasis is prevalent in 82 countries and has infected about 12 million people worldwide.[1] Recently, more than a thousand *Leishmania* strains have been isolated and identified from patients co-infected with HIV, which can exhibit wider drug-resistance, and, interestingly, the majority of these cases where found on individuals in industrialized countries such France, Spain and Italy.[2] The United States of America and Canada, countries where leishmaniasis is not endemic, face canine leishmaniasis as a public health problem;[3] furthermore, in the year 2004, 522 cases of cutaneous leishmaniasis where confirmed by the US Department of Defense, from military personnel who served in Southwest/Central Asia.[4] With no available vaccine, the only control intervention chemotherapy against leishmaniasis involves the use of highly toxic pentavalent antimonials, developed decades ago and for which resistance has been extensively reported.[5] Amphotericin B is used alternatively as second-line drug; however it is highly toxic and expensive for routine use in resource-poor countries.[1e] Clearly, the development of new effective drugs with high selectivity, minimum side effects and low manufacture costs is urgently required. The economical, facile and rapid synthesis of chalcones,[6a] make them attractive as potential drug candidates to fight leishmaniasis and some other of the so-called neglected diseases that affect the populations of many countries in the Third World.

Chalcones exhibit a wide variety of pharmacological activities, among them their anticancer, antibacterial and antiprotozoal are remarkable.[6] Recently, we have reported the synthesis, cytotoxicity and in vitro anti-*Trypanosoma cruzi* activity of 28 new chalcones featuring hydroxyl and allyoxy moieties on the ring A.[7] On this communication, we want to report the in vitro anti-Leishmania activity of 43 chalcones and the in vivo study of five of the ones that exhibited the highest parasite-development inhibition and the lowest cytotoxicity, namely, **21**, **38**, **41**, **42** and **43**.

The general method for the synthesis of chalcones is summarized in Scheme 1; the preparation of compounds **1**–**40**, has been described previously.[7] Synthesis of chalcones **41**–**43** was accomplished using previously reported methodology.[8] All synthetic compounds are described on Table 1. To the best of our knowledge this is the first report for preparation of 13 compounds (**25**, **27**, **32**–**40** and **43**). In this present work we have evaluated the activity of

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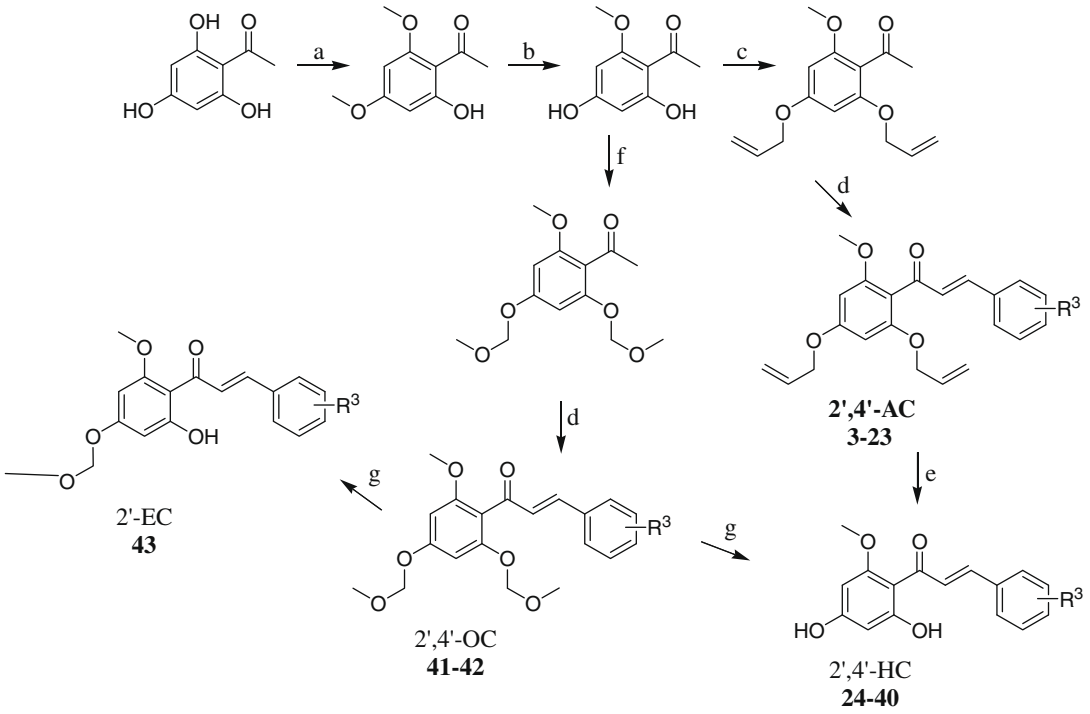


Scheme 1. Reagents and conditions: (a) K_2CO_3, $(CH_3)_2SO_4$, $(CH_3)_2CO$, 65 °C, 6 h; (b) $AlCl_3$, benzene, reflux, 1 h; (c) K_2CO_3, allyl bromide, DMF, rt, overnight; (d) Claisen–Schmidt aldol condensation of an acetophenone with an aromatic aldehyde, KOH, H_2O, CH_3OH, rt, 1–48 h; (e) K_2CO_3, catalytic $Pd(PPh_3)_4$, MeOH, 60 °C, 1 h; (f) K_2CO_3, $(CH_3)_2CO$, CH_3OCH_2Cl, reflux, 3 h; (g) HCl 3 N, reflux, CH_3OH, 30 min.

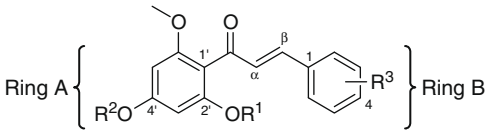
43 substituted chalcones against axenic amastigotes of *Leishmania amazonensis* (Table 1), and the anti-*L. braziliensis* and anti-*L. peruviana* activity of nine chalcones, using macrophages infected with the corresponding parasites (Table 2). As shown in Table 1, the great majority (74%) of the synthesized compounds exhibited an IC_{50} value below 20 μM, while 21 of them (49%) an IC_{50} value below 10 μM; more interestingly, nine of the most active compounds (**21**, **26**, **30**, **31**, **38**, **40**–**43**) showed a selectivity index (SI) greater the 10. Among the two largest series of chalcones (2′,4′-diallyloxy-6′-methoxychalcones, 2′,4′-AC, **3**–**23** and 2′,4′-dihydroxy-6′-methoxychalcones, 2′,4′-HC, **24**–**40**), it was the 2′,4′-HC chalcone series, which showed the better selectivity against the axenic amastigotes of *L. amazonensis*, despite not being the series with the most active derivatives.

The anti-Leishmania activity of chalcones with different substitution patterns have been previously reported.[6] Herein, we present for the first time, the anti-Leishmania activity of 2′,4′-AC; 2′,4′-HC; 2′,4′-OC and 2′-EC. Among compounds **1**, **5**, **26** and **41**, which differ only on the substitution pattern on ring A, it can be observed that having a methoxymethyl substituent on the 2′,4′-position not only maintains the activity of the molecule, but also greatly enhances its selectivity against the parasite, when compared to its 2′,4′-allyoxy and 2′,4′-hydroxy analogs. This observation suggests that there exists considerable tolerance for the size and substitution pattern on ring A. In the same way, compounds **4**, **7**, **8**, **10**, **12**, **18** and **23**, all of them having 2′,4′-diallyloxy moieties on the ring A, resulted inactive (IC_{50} values >80 μM) while their corresponding 2′,4′-dihydroxy substituted counterparts, compounds **25**, **28**, **29**, **31**, **33**, **38** and **40** showed IC_{50} values ranging from 15.7 to 1.1 μM. These results are in agreement with the results reported by Liu and co-workers,[6b] which showed that the anti-Leishmania activity of chalcones is enhanced by the presence of polar 2′ and 4′-hydroxy groups. However, when comparing the rest of active 2′,4′-AC series (**3**, **5**, **6**, **9**, **11**, **13**–**16**) against their corresponding 2′,4′-HC series (**24**, **26**, **27**, **30**, **32**, **34**–**37**), all of them bearing electron donating or electron withdrawing groups on the ring B, it seems clear that the anti-parasitic activity for this set of compounds is independent of the substitution pattern on the ring A, since the anti-Leishmanial activity is conserved for each analog pair. This appreciation is also applicable for inactive compounds such **19** and **39**, in which it can also be seen that the conjugation of the double bond on the α,β-unsaturated bridge results on the lost of the bioactivity.

Table 2 shows the bioactivities of the nine compounds which showed the highest SI on the axenic amastigotes assay. From this macrophage-infected model, it can be observed that, in general, *L. peruviana* was the species of parasites which showed the strongest resistance towards all chalcones, whereas *L. braziliensis* and *L. amazonensis* seemed to respond differently depending on the type of chalcone administered. Compound **41** showed an interesting selectivity against *L. braziliensis*. Although it showed selectivity against the *L. amazonensis* species (29 μM), compound **31**—which resulted highly selective on the axenic amastigote assay—resulted inactive against *L. braziliensis* and *L. peruviana* (237 and 290 μM, respectively). On the other hand, compound **21**, containing a pyridinyl moiety, seemed to exert the highest bioactivity against all *Leismania* species with values ranging from 0.9 to 4.0 μM.

Considering these in vitro results, as well as their synthetic and physicochemical characteristics; we started an in vivo study using compounds **21**, **38**, **41**, **42** and **43**. During the preparation of the 2′,4′-HC series, we did not find the isomeric flavanone that could arise as a condensation product for any of the compounds in this series. For the in vivo assay, a larger amount of sample was needed, and in this regard the synthesis of compounds **26**, **30** and **31** was not as readily simple as for compounds **21**, **41**–**43**. Therefore, besides compounds **26** and **30** showed moderate activities on the macrophage-infected model, they were not selected for the in vivo screening. However, the chemical functionalities on ring B for **26** and **30**, are in close relation to those on compounds **41**–**43**, furthermore; the solubility of the later is several times greater in DMSO. The in vivo results are summarized on Table 3. From these results, we can conclude that none of the five tested compounds was as effective as the positive control (Gluc) to reduce

Table 1
In vitro activity of compounds **1–43** against axenic amastigotes of *L. amazonensis* and macrophage



Compds	Ring A	Ring B	IC_{50}[a] (μM)		
			L. amazonensis	Macrophage	SI[b]
1	2′-Hydroxy,4′-methoxy	4-Methoxy	17.2 ± 12.3	nd[c]	—
2		3,4-Methylenedioxy	>300	—	—
3	2′,4′-Diallyloxy	Benzyl	9.4 ± 1.5	14.8 ± 2.5	1.6
4		4-Methyl	79.8 ± 5.2	—	—
5		4-Methoxy	5.5 ± 1.0	18.7 ± 0.7	3.4
6		3-Methoxy	8.7 ± 0.8	13.1 ± 1.1	1.5
7		4-Hydroxy	85.2 ± 3.3	—	—
!8		4-Hydroxy-3-Methoxy	107.2 ± 5.1	—	—
9		3,4-Methylenedioxy	6.6 ± 4.2	nd	—
10		2,4-Dimethoxy	134.5 ± 9.7	—	—
11		3,4,5-Trimethoxy	8.2 ± 3.9	12.0 ± 0.6	1.5
12		4-Trifluoromethyl	127.6 ± 5.6	—	—
13		4-Chloro	9.4 ± 4.3	13.0 ± 2.0	1.4
14		4-Fluoro	9.5 ± 1.8	13.8 ± 0.9	1.5
15		2-Fluoro	9.5 ± 0.8	13.3 ± 5.3	1.4
16		2-Bromo	7.9 ± 2.4	5.4 ± 1.0	0.7
17		4-Nitro	8.3 ± 1.0	13.2 ± 0.4	1.6
18		4-Bromo-3,5-Diallyloxy	>180	—	—
19		Cinnamyl	75.2 ± 10.3	—	—
20		4-Pyridinyl	0.6 ± 0.1	nd	—
21		2-Pyridinyl	1.1 ± 0.3	12.5 ± 0.8	11.4
22		2-Pyrrolyl	94.3 ± 11.4	—	—
23		2-Funanyl	>300	—	—
24	2′,4′-Dihydroxy	Benzyl	15.9 ± 1.7	27.0 ± 2.0	1.7
25		4-Methyl	7.4 ± 1.9	16.5 ± 4.8	2.2
26		4-Methoxy	10.0 ± 0.1	166.2 ± 4.7	16.6
27		3-Methoxy	12.0 ± 0.2	17.6 ± 2.9	1.5
28		4-Hydroxy	15.7 ± 3.6	21.7 ± 0.7	1.4
29		4-Hydroxy-3-Methoxy	14.9 ± 1.7	nd	—
30		3,4-Methylenedioxy	4.1 ± 1.2	157.2 ± 0.9	38.3
31		2,4-Dimethoxy	6.4 ± 0.9	>300	>50
32		3,4,5-Trimethoxy	9.7 ± 1.2	nd	—
33		4-Trifluoromethyl	8.6 ± 2.0	17.1 ± 1.8	2.0
34		4-Chloro	11.2 ± 1.6	19.0 ± 4.2	1.7
35		4-Fluoro	11.8 ± 0.3	28.8 ± 2.6	2.4
36		2-Fluoro	11.8 ± 0.5	16.0 ± 1.1	1.4
37		2-Bromo	10.6 ± 2.4	14.6 ± 5.5	1.4
38		4-Bromo-3,5-Diallyloxy	1.1 ± 0.1	11.5 ± 2.0	10.5
39		Cinnamyl	52.6 ± 4.8	—	—
40		2-Funanyl	4.6 ± 1.1	384.3 ± 13.2	83.5
41	2′,4′-Dimethoxy methyl	4-Methoxy	1.3 ± 0.2	>250	>200
42		3,4-Methylenedioxy	11.7 ± 4.1	142.9 ± 11.0	12.2
43	2′-Hydroxy-4′-methoxymethyl	3,4,5-Trimethoxy	6.2 ± 0.7	132.5 ± 31.3	21.4
	Amphotericin B		0.1 ± 0.01	5.3 ± 0.2	76.0

[a] IC_{50}: concentration that produces 50% inhibitory effect.
[b] SI: selectivity index = $IC_{50,\ macrophage}/IC_{50,\ L.\ amazonensis}$.
[c] Not determined.

Table 2
Activity of chalcones against macrophages infected with three *Leishmania* species (values in μM)

Compds	*L. amazonensis* Lma CL1	*L. braziliensis* PER006	*L. peruviana* LCA08
21	0.9	1.4	4.0
26	14.3	7.6	23.0
30	16.6	8.6	19.7
31	28.5	237.0	290.2
38	4.1	14.4	nd[b]
40	16.9	30.4	34.2
41	12.1	5.2	110.8
42	24.0	17.2	34.8
43	29.7	13.1	19.3
Amp. B[a]	0.4	0.1	0.1

[a] Amphotericin B.
[b] Not determined.

the lesion diameter (mice's footpad measured with a caliper). However, since these results depend on a series of parameters such as immune response, inflammation process and parasite virulence—which are not proportional to the parasite load and the parasite burden—we completed the measure of mice's footpad by counting the *L. amazonensis* amastigotes in the foot tissue using a fluorescent probe.

From this count, compounds **42** and **43** (administered to the infected mice in a concentration almost seven times lower than the positive control) showed high reduction of the parasite burden, with $P = 0.0004$ and $P = 0.0019$, respectively, after the initial four weeks of treatment, meaning a reduction of the parasite burden of 92% and 74%, respectively. These results were further confirmed by the experiment at the seventh week in which they showed a 35% and 41% reduction of parasite burden, respectively.

Table 3
In vivo activity of chalcones on *L. amazonensis*-infected BALB/c mice ($n = 10$)[a]

Compds	Lesion diameter (mm)[b] after 4 weeks	Reduction of parasite burden (%)	
		After 4 weeks	After 7 weeks
Control	2.8 ± 0.4	—	—
21	3.0 ± 0.3	−4	25[d]
38	3.1 ± 0.3	9	8[d]
41	2.8 ± 0.1	11	17
42	2.9 ± 0.2	92	35
43	3.5 ± 0.5	74	41
Gluc.[c]	2.1 ± 0.1	100	100

[a] Effect of treatments after eight intralesional inoculations.
[b] Compounds administrated at 5 mg/kg/day.
[c] *N*-methylglucamine antimoniate, administrated at 33 mg/kg/day.
[d] Compounds **21** and **38** were analyzed after 6 weeks treatment.

Compounds **42** and **43** did not exhibit any cutaneous toxicity at the tested doses and they may not exhibit a reduction of the lesion diameter, probably due to their lack of anti-inflammatory activity.

In conclusion we have screened 43 polysubstituted chalcones against *L. amazonensis* amastigotes and further tested the most selective against a macrophage-infected model using the three most infectious parasites prevalent in Peru. After this large in vitro screening, five of the best compounds were selected for in vivo analysis, resulting on the identification of two chalcones with high anti-parasitic potential.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.11.033.

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