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FULL PAPER

Antiprotozoal Activity of Xanthone Derivatives

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Abstract:

A series of xanthone derivatives containing different side chains, including ω-bromo and ω-aminoalkoxylxanthones (with linear alkoxy chains of 3, 4 and 5 carbon atoms and methyl, propyl, *tert*-butylamino and piperidinyl moieties), synthesized from the natural xanthone lichexanthone were tested for their antiprotozoal activities against *Leishamania braziliensis*, *L. major* and *Trypanosoma cruzi* (extra and intracellular forms). The ω-aminoalkoxylxanthones showed good antileishmanial activity, with IC_{50} ranging from 62.8 to 0.1 μM for promastigotes and 119.3 to 2.4 μM for amastigotes. In general, compounds with longer alkyl chains and *tert*-butylamino moiety showed better activity. The cytotoxicity on VERO cells was also described for some derivatives. Compound **15** (*tert*-butylaminobutyloxy side chain) was the most active on promastigote forms (IC_{50} 0.1 μM), compound **16** (*tert*-butylaminopentyloxy side chain) sowed the best activity for amastigotes (IC_{50} 2.4 μM) and derivative **13** (dipropylaminopentyloxy side chain) was the most selective (selectivity index of 9.4). Regarding trypanocidal activity, **16** and other groups of derivatives, (ω-bromoalkoxylxanthones, prenyl and epoxyl side chains) had moderate to good activity on *T. cruzi* trypomastigotes, with IC_{50} ranging from 30.6 to 4.1 μM, while only two ω-aminoalkoxylxanthones (**10** and **11**) were weakly active against *T. cruzi* amastigotes.

Keywords: lichen; *Leishmania* spp; structural modification; *Trypanosoma cruzi*; xanthone

1. Introduction

Leishmaniasis and Chagas Disease belong to a group of diseases called neglected tropical diseases, known to affect people among the poorest economic nations, causing over 500,000 deaths per year [1].

According to estimates of the World Health Organization (WHO), 350 million individuals are living at risk with *Leishmania* spp parasites. Several species of this protozoan parasite cause a broad range of pathologies collectively called leishmaniasis, a complex disease with an estimated prevalence of 1.3 million new cases and 20,000 deaths every year. *Leishmania major* and *L. braziliensis,* the two species assayed in this study, cause cutaneous leishmaniasis, and the last can also cause the mucocutaneous form of the disease with destructive lesions involving secondary cartilage and mucous membranes [2]. The therapeutic alternatives comprise a small group of drugs: amphotericin B, miltefosine, paromomycin and pentavalent antimonials, with low efficacy to some parasite´s lines, high toxicity and high costs [3-5].

Chagas' disease is a parasitic infection widely distributed throughout Latin America, with devastating consequences in terms of human morbidity and mortality. Caused by the protozoan parasite *Trypanosoma cruzi*, Chagas' disease affects about 8 million people, leading to 10,000 deaths and 300,000 new cases every year. The existing drug therapy, reduced to benznidazole and nifurtimox, suffers from a combination of drawbacks including poor efficacy, resistance and serious side effects [6-8].

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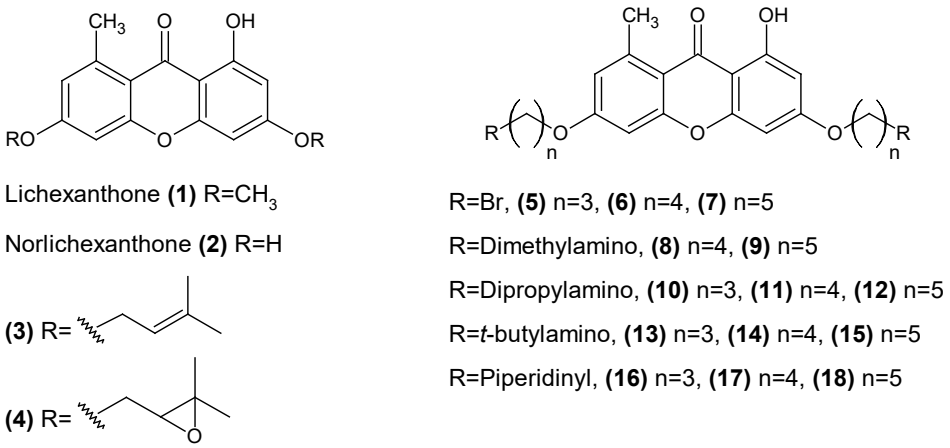
Xanthones are known to show a wide range of biological activities such as antibacterial, antidiabetic, antiplasmodial, human cancer cell line growth inhibition, antihypertensive and vasorelaxing, cardiovascular protection, anti-HIV, antioxidant [9, 10]. Several xanthones derived from natural sources, as well as synthetic xanthone derivatives were found to be promising compounds to treat parasitic infections [11-15].

The mode of action towards *Plasmodium* and *Leishmania* parasites was discussed by Kelly et al. [11, 16] and Riscoe et al. [17] and, in both cases, it was related to xanthones ability in complexing with heme. For *Plasmodium* it implies in inhibition of heme polymerization and further detoxification of harmful hematin [17]. *Leishmania* and *Trypanosoma* parasites are partially or totally incapable of heme biosynthesis and must scavenge for heme from the host cell [18]. Given that heme is essential to the survival of *Leishmania*, drugs which interfere with parasite heme acquisition processes may prove useful in treatment of the leishmaniases. Once all trypanosomatids require heme or pre-formed porphyrins for growth due to a lack of several key enzymes in the heme biosynthetic pathway [11], we've decided to test a series of xanthone derivatives, including ω-aminoalkoxylxanthones, synthesized from the natural product lichexanthone **(1)** against *L. major, L. braziliensis* and *T. cruzi* in order to evaluate their antiparasitic potential towards these parasites.

2. Results and Discussion

Isolation of lichexanthone **(1)** and synthesis of derivatives **2** to **19** (Figure 1) were carried out as described by Micheletti et al. [19, 20].



Lichexanthone **(1)** R=CH_3

Norlichexanthone **(2)** R=H

(3) R=

(4) R=

R=Br, **(5)** n=3, **(6)** n=4, **(7)** n=5

R=Dimethylamino, **(8)** n=4, **(9)** n=5

R=Dipropylamino, **(10)** n=3, **(11)** n=4, **(12)** n=5

R=*t*-butylamino, **(13)** n=3, **(14)** n=4, **(15)** n=5

R=Piperidinyl, **(16)** n=3, **(17)** n=4, **(18)** n=5

Figure 1. Chemical structures of evaluated compounds.

Compounds **1** to **18** were tested for their *in vitro* antiparasitic activity against *L. major*, *L. braziliensis* and *T. cruzi*, once antiparasitic potential of xanthones, especially ω-aminoalkoxyl derivatives is widely discussed [11, 17]. Results are summarized in Table 1 (mean values ± SD of three experiments, for intracellular forms).

Among ω-aminoalkoxyl xanthones, it´s interesting to notice that, overall, the derivatives containing *N*-dimethyl (**8** and **9**) and *N*-*t*-butyl (**13-15**) moieties, and the ones bearing longer carbon chains were more active against all tested *Leishmania* parasites. Riscoe et al. [17], based in molecular modeling studies, suggested that a chain length in the range of 4 to 6 carbon atoms would be optimal to permit a closer association between the xanthone side chain nitrogen and the carboxylate groups of heme.

For promastigote forms, infectious flagellated cells that exist in the digestive tract of their sandfly vector [21], almost all ω-aminoalkoxylxanthones were more active than the positive control, amphotericin B. Most of the evaluated compounds were more active on *L. braziliensis*, but the IC_{50} value for amphotericin B against *L. major* is higher, suggesting this parasite could be more resistant, what may

explain this observation. For *L. braziliensis*, the most active derivative was **14**, bearing a four-carbon spacer between N and O, with IC_{50} of 0.1 μM, and for *L. major*, derivative **15**, with a five-carbon spacer, showed the best activity.

Once compounds were more active on *L. braziliensis* promastigotes, this species was also used for an assay on amastigotes, immotile intracellular forms in the cells of their host's mononuclear phagocytic system [21], and most derivatives kept low IC_{50} values. Compounds **8**, **10**, **13**, **14** and **16** were less active against the intracellular form, while **3**, **11**, **12**, **15**, **17** and **18**, proved more active, being **15** the most active one, with an IC_{50} of 2.4 μM. As information about cytotoxicity on VERO cells were also available for some compounds [22], the selectivity indexes (SI = IC_{50} over VERO cells/IC_{50} against *Leishmania*) were calculated. When the SI is evaluated, compound **12** is highlighted, and despite its higher IC_{50} value (13.4 μM), the selectivity index is 9.4, against a selectivity index of 6.5 to compound **15**.

Table 1. Antileishmanial, trypanocidal and cytotoxic activities for compounds **1-18** (IC_{50} values given in μM).

Compound	IC_{50} (μM)					
	L. major	*L. braziliensis*		*T. cruzi*		*VERO cells* [22]
	promastigote	*promastigote*	*amastigote* (± SD)	*trypomastigote*	*amastigote* (± SD)	
1	n.a	n.a	n.t	n.a	n.t	n.t
2	n.a	n.a	n.t	n.a	n.t	n.t
3	n.a	n.a	48.1± 2.3	11.2	n.a	n.t
4	n.a	41.8	39.5 ± 0.44	13.7	n.a	n.t
5	n.a	145.4	n.a	n.a	n.a	n.t
6	20.5	n.a	n.a	n.a	n.a	n.t
7	n.a	n.a	n.a	22.9	n.a	n.t
8	n.a	4.5	15.1 ± 6.3	n.a	n.a	n.t
9	9.5	5.8	6.4 ± 8.1	n.a	41.6 ± 1.0	n.t
10	35.1	21.7	119.3 ± 2.9	n.a	44.4 ± 0.5	n.t
11	62.8	25.9	12.7 ± 2.2	n.a	n.a	50.1
12	11.5	21.5	13.4 ± 2.6	n.a	n.a	126.8
13	10.8	3.0	8.67	n.a	n.a	13.0
14	9.1	0.1	7.1 ± 7.7	n.a	n.a	13.1
15	5.4	7.3	2.4 ± 5.7	4.1	n.a	15.7
16	6.4	8.8	50.2 ± 2.5	n.a	n.a	n.t
17	23.0	17.6	7.4 ± 2.7	n.a	n.a	12.9
18	12.6	22.9	6.4 ± 5.7	30.6	n.a	15.9
Amphotericin B	37	22	-	-	-	
Benznidazole	-	-	-	31	-	

n.t = not tested; n.a = not active; SD= standard deviation.

Kelly et al. [11] reported IC_{50} values of 0.2 to 0.03 μM for 3,6-bis-ω-diethylaminoalkoxylxanthones evaluated against *L. mexicana*, and observed a correlation between activity and carbon chain length, where longer chains presented better activities, what was also observed for the compounds tested in this study, in general. The IC_{50} values were also higher, however once *Leishmania* species are different, resistance profile can be different too. Ignatushchenko and co-workers [23] suggest that xanthones with hydroxyl groups in *peri* position (1 or 8) are less active on *Plasmodium* that the corresponding isomers with different substitution pattern probably by decreasing interaction of the xanthone carbonyl to the iron atom of metalloporphyrins because of the hydrogen bond established between carbonyl and hydroxyl groups, which could be extrapolated to *Leishmania*. However, Portela et al. [24] presented a series of xanthone derivatives active against *Plasmodium falciparum* with a nitrogen atom at position 1, where the possibility of hydrogen bonding with the keto carbonyl also exists.

Among the other group of xanthones tested (**1-7**), **6** was slightly active on *L.* major promastigotes, **4** showed weak activity on *L. braziliensis* promastigotes, while **3** and **4** were active against amastigotes. These findings reinforce the importance of the side carbon chain length together with the presence of a basic group [17].

Results for antiparasitic effect against *T. cruzi* did not follow the pattern achieved for *Leishmania* species. Regarding the assay on trypomastigotes, the infecting bloodstream forms [6], only two ω-aminoalkoxylxanthones were active. Compound **15** showed the best activity, with IC_{50} of 4.1 μM, and **18**, containing a piperidinyl moiety and a five-carbon spacer between N and O, was weakly active (IC_{50} of 30.6 μM). This fact should indicate that the mode of trypanocidal activity is different from that for leishmanicidal activity. Other xanthones showed good to moderate activity: the *O*-prenylated derivative **3**, the corresponding epoxide **4** and the ω-bromoalkoxylxanthone **7**. For intracellular amastigote forms, only derivatives **9** and **10** were active. There are some works describing trypanocidal activity of xanthones [12,25-29], all of them related to natural products isolated from plants and fungi, and this is first report of trypanocidal activity for a series of synthetic derivatives. Some of the already studied compounds are prenylated [25-28], however, the substitution patterns are distinct when compared with **3**.

3. Material and Methods

3.1 Isolation and synthesis

Isolation of lichexanthone **(1)** and synthesis of derivatives **2** to **18** were carried out as previous described procedures [19, 20].

3.2 Antiparasitic assays

3.2.1. Leishmanicidal activity

First, promastigote forms of *L. braziliensis* and *L. major* were cultivated in 199 (LGC) culture medium supplemented with 5% of calf newborn serum, penicillin and streptomycin. After 6 days of initial inoculation, promastigote forms ($1x10^7$/mL) were obtained and incubated in 96-well microtiter plates. The compounds were added at different concentrations (0.5, 2.0, 8.0 and 32 μM) and plates were evaluated after 24 and 72h by colorimetric MTT oxidation technique. The positive control used was amphotericin B (at the same concentration of tested compounds), while dimethyl sulfoxide at 1% in physiologic solution was used as negative control.

Active compounds were also tested against amastigote forms of *L. braziliensis*. Promastigote forms ($5x10^6$/mL) were obtained as described above. These parasites were then added to a 96-well microplate previously incubated with J774 macrophages (2×10^5 cells/mL) and incubated overnight at 37 °C, 5% CO_2. After incubation, the culture medium (RPMI-1640, GIBCO) was removed and the infected cells washed to remove promastigote forms that did not infect macrophages. RPMI medium containing different concentrations of the substances (0.5; 2.0; 8.0 and 32.0 μM) was then added in triplicate, and the plate was again incubated under the same conditions for 72 hours. After this period, the cells were trypsinized and SYTO 9® stain (Life Technologies) was added to the culture medium, according to the manufacturer's recommendations, and the parasites were counted in an image-based cytometer (TALI - Life Technologies). As negative control was used RPMI 1640 medium containing DMSO in the same concentration used in the tests.

3.2.2. Trypanocidal activity

The trypanocidal activity of the compounds was evaluated on both trypomastigotes and amastigotes forms of clone B5 of the *T. cruzi* CL Brener *LacZ* strain.

For the trypomastigotes forms LLC-MK2 cells were used for parasite´s cultivation cultured in RPMI 1640 medium (Sigma) supplemented with 5% fetal bovine serum (Cultilab), penicillin G (25 IU/mL), streptomycin (25 μg/mL) and ciprofloxacin (10 μg/mL) at 37 °C in a humidified atmosphere containing 5% CO_2. For the assay, LLC-MK2 (ATCC) cells were resuspended in RPMI 1640 medium (Sigma) with 5% fetal bovine serum for 24 h in 96-well plates. Then, the cells were infected with 5.0×10^5 trypomastigotes forms and the plates were incubated for 96 h.

Free trypomastigotes forms in the supernatant were collected, centrifuged and the number of the parasites were adjusted to 1 x 10^6 parasites/mL in a 96-well plate. Thus, compounds were added to give final concentrations 0.5; 2.0, 8.0 and 32 μM and incubated at 4°C for 24 h.

Active compounds were also evaluated for the amastigote forms of the parasite. Cells from the LLCMK2 lineage were plated at a concentration of 5x10^4 cells/mL. Trypomastigote forms of the parasite were added at a concentration of 5×10^5 parasites/mL and placed in the incubator at 37°C with 5% CO_2 for 24 hours. After the incubation period, the extracellular trypomastigotes forms present were removed by successive washes with PBS, remaining only the intracellular amastigote forms. Compounds were added at different concentrations (0.5; 2.0, 8.0 and 32 μM) and incubated for 72 hours.

For both forms of the parasite, after treatment with the compounds, 50 μL of CPRG (chlorophenol red β-D-galactopyranoside, 400 μM in 0.3% Triton X-100, pH 7.4) solution was added and the plates incubated at 37.0 °C for 6 hours and absorbance read at 595 nm. Assays were performed in triplicate for amastigotes. Benznidazole was used as a positive control in the same concentrations as the substances, and DMSO (0.2%) as a negative control for both assays.

4. Conclusions

A series of xanthones, including *O*-prenylated, epoxide, brominated and nitrogenated derivatives were tested for their antiprotozoal effects against *L. braziliensis, L. major* and *T. cruzi* (extra and intracellular forms). For leishmanicidal activity, nitrogenated derivatives were more active, and showed in general some selectivity for *L. braziliensis*, being in addition tested against intracellular forms this parasite. Results are encouraging since many compounds showed low IC_{50} values. However, their antiprotozoal activities were not extended to *T. cruzi*, and the active substances presented different substituents, like *O*-prenyl, epoxide and brominated side chains. Once these parasites cause severe diseases, with high mortality rate, the results presented here are of great importance and add to the efforts in the search for new compounds with antiparasitic activity.

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