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Gibbilimbol analogues as antiparasitic agents—Synthesis and biological activity against *Trypanosoma cruzi* and *Leishmania* (*L.*) *infantum*

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ABSTRACT

The essential oils from leaves of *Piper malacophyllum* (Piperaceae) showed to be mainly composed by two alkenylphenol derivatives: gibbilimbols A and B. After isolation and structural characterization by NMR and MS data analysis, both compounds were evaluated against promastigote/amastigote forms of *Leishmania* (*L.*) *infantum* as well as trypomastigote/amastigote forms of *Trypanosoma cruzi*. The obtained results indicated that gibbilimbol B displayed potential against the tested parasites and low toxicity to mammalian cells, stimulating the preparation of several quite simple synthetic analogues in order to improve its activity and to explore the preliminary structure–activity relationships (SAR) data. Among the prepared derivatives, compound **LINS03003** (*n*-octyl-4-hydroxybenzylamine) displayed the most potent IC_{50} values of 5.5 and 1.8 μM against amastigotes of *T. cruzi* and *L.* (*L.*) *infantum*, respectively, indicating higher activity than the natural prototype. In addition, this compound showed remarkable selectivity index (SI) towards the intracellular forms of *Leishmania* (SI = 13.1) and *T. cruzi* (SI = 4.3). Therefore, this work indicated that preparation of synthetic compounds structurally based in the bioactive natural products could be an interesting source of novel and selective compounds against these protozoan parasites.

Leishmaniasis and American trypanosomiasis (Chagas disease) are tropical diseases caused by protozoan parasites belonging to *Leishmania* and *Trypanosoma* genus.[1–4] Nowadays there is a reduced number of drugs to the treat these diseases, which include highly toxic compounds such as pentamidine, amphotericin B, miltefosine and nitroheterocyclic compounds (benznidazole and nifurtimox), demonstrating the urgent necessity of novel and alternative treatments.[5–7] Prospection of pharmacologically active metabolites from plant species could be considered an interesting approach to discovery of prototypes to be used in the development of the new drugs to the treatment of parasitic diseases, mainly those used in the ethnopharmacological point of view.[8–10] In a previous work,[11] our group reported the antiparasitic activity of gibbilimbol B, an alkylphenol derivative isolated from leaves of *Piper malacophyllum* (Piperaceae) against promastigotes and amastigotes of *Leishmania* (*L.*) *infantum*, as well as *Trypanosoma cruzi* trypomastigotes. Our previous studies demonstrated that gibbilimbol B affected the permeability of plasma membrane of *L.* (*L.*) *infantum*, leading to cell death, a similar effect observed by the clinical used drug amphotericin B. This action may be attributed to its amphipathic characteristic that resembles membrane phospholipids, where the phenolic hydroxyl can be directed to the aqueous layer and the alkyl chain to the hydrophobic environment of membrane.

In continuation to our studies with *P. malacophyllum*, the essential oil from leaves showed to be composed by additional amounts of gibbilimbol B as well as by its isomeric derivative gibbilimbol A (Fig. 1). After several chromatographic steps,[12] both compounds were separated and their antiparasitic activities were individually evaluated. As can be seen in Table 1, gibbilimbol A displayed weak activity against amastigote forms of *L.* (*L.*) *infantum* and trypomastigote forms of *T. cruzi*, with IC_{50} of 135.7 and 102.5 μM, respectively. Otherwise, gibbilimbol B, an isomeric derivative of gibbilimbol A (with double bond at C-4′ instead of C-3′) displayed higher activity against the tested parasites and low toxicity to mammalian cells. The result suggests the presence of unsaturation close to aromatic ring leads to improved activity, and thus other

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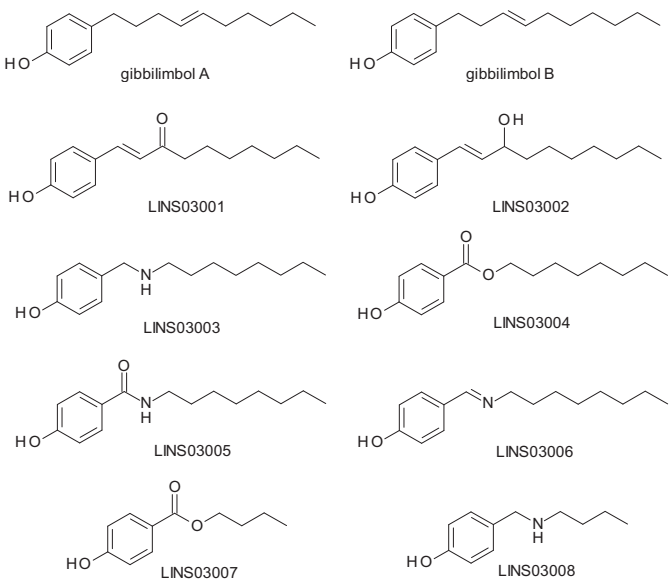

Figure 1. Structures of gibbilimbols A/B and related analogues (**1**–**8**).

functional groups in this region should be explored. Therefore, this work aimed the preparation of synthetic compounds structurally related to gibbilimbol B and the evaluation of the antiparasitic activity against *L.* (*L.*) *infantum*/*T. cruzi*, extracellular and intracellular forms and mammalian cytotoxicity, as well as to explore the structure–activity relationships (SAR).

Considering the alkyl side chain nature of gibbilimbol derivatives and the previous reported targeting ability to *Leishmania* plasma membrane,[11] interactions with specific membrane components such as ergosterol could be plausible effect of these compounds. Thus, eight analogues of gibbilimbols (LINS0300**X**, **1**–**8**) were designed (Fig. 1) to evaluate possible interactions by hydrogen-bonding and the role of the length of side chain (alkenyl or alkyl) in the activity. Polar functional groups were inserted in this moiety to provide additional interaction sites, so derivatives possessing carbonyl (**1**), hydroxyl (**2**) and amine (**3**) groups were designed. As metabolic deactivation is also desirable in a future drug to avoid excessive toxicity to mammalian cells,[13] ester (**4**), amide (**5**) and imine (**6**) derivatives were also proposed, since they can be easily hydrolyzed in vivo. Moreover, lower homologues of these compounds (**7** and **8**) were also synthesized and evaluated.

Compounds **1**–**8** were synthesized according to the Schemes 1 and 2, through classical methods which conducted to excellent yields.[14] Compound **1** was obtained through aldol condensation of 4-hydroxybenzaldehyde and 2-nonanone under basic conditions.[15] This compound was then used to obtain **2** by reduction of carbonyl group using sodium triacetoxyborohydride (STAB) prepared in situ prior to reduction.[16] Interestingly, only the product of 1,2-reduction was observed. Generally α,β-unsaturated ketones can be reduced to both 1,2- and 1,4-reduction products when reacted with borohydride.[17] However, the distribution of these products is variable. It is well known STAB is a 1,2-selective agent in the α,β-unsaturated aldehydes reduction. However it is not a good reducing agent for α,β-unsaturated ketones.[18] Possibly the extended conjugation to the aromatic ring played the role to obtain selectively the 1,2-reduction product **2**.

Table 1
Antiparasitic activities of gibbilimbols A/B, and of the synthetic derivatives **1**–**8** against trypomastigote/amastigote forms of *T. cruzi* and promastigote/amastigote forms of *L.* (*L.*) *infantum*

Compounds	IC_{50} μM (95% CI)				CC_{50} μM (95% CI)	SI[b]			
	T. cruzi trypomastigotes	*T. cruzi* amastigotes	*L. (L.) infantum* promastigotes	*L. (L.) infantum* amastigotes	NCTC cells[a]	TCT	TCA	LIP	LIA
1	11.8 (10.1–13.1)	22.5 (19.1–26.5)	82.7 (74.3–92.2)	NA	82.8 (66.5–103.1)	7.0	3.7	1.0	—
2	6.1 (5.5–6.7)	NA	125.1 (90.3–174.4)	NA	49.5 (32.9–74.4)	8.1	—	0.4	—
3	17.0 (14.0–20.8)	5.5 (4.6–6.5)	28.6 (25.3–32.6)	1.8 (1.1–2.9)	23.5 (15.5–82.9)	1.4	4.3	0.8	13.1
4	4.5 (3.7–5.4)	NA	123.4 (114.0–133.0)	NA	130.3 (107.9–157.4)	29.0	—	1.1	—
5	26.5 (22.6–31.2)	NA	45.1 (38.8–52.3)	NA	45.4 (27.3–75.3)	1.7	—	1.0	—
6	NA	NA	19.9 (17.5–22.6)	NA	99.2 (79.3–124.3)	—	—	5.0	—
7	NA	26.4 (20.4–33.3)	NA	NA	167.8 (98.3–286.4)	—	6.4	—	—
8	NA	NA	31.6 (24.1–41.5)	NA	>300	—	—	9.5	—
Gibbilimbol A	102.5 (74.5–122.7)	NA	NA	135.7 (92.1–163.5)	224.6 (201.3–256.9)	2.2	—	—	1.7
Gibbilimbol B[c]	75.3 (62.3–90.9)	NA	100.4 (82.6–121.8)	94.9 (73.2–123.2)	254.1 (217.9–296.3)	3.4	—	2.5	2.7
Miltefosine	ND	ND	16.7 (13.0–21.5)	16.4 (15.4–17.4)	241.4 (206.9–281.6)	—	—	14.5	14.7
Benznidazole	440.7 (406.1–478.3)	230.3 (176.2–301.1)	—	—	269.9 (414.9–532.1)	0.6	1.2	—	—

IC_{50}: 50% inhibitory concentration.
NA: not active.
ND: not determined.
[a] CC_{50}: 50% cytotoxic concentration.
[b] SI: selectivity index calculated from CC_{50}/IC_{50} (TCT: *T. cruzi* trypomastigotes, TCA: *T. cruzi* amastigotes, LIP: *L.* (*L.*) *infantum* promastigotes, LIA: *L.* (*L.*) *infantum* amastigotes).
[c] Available from Oliveira et al.[11]