Antibacterial and Antileishmanial Activity of 1,4-Dihydropyridine Derivatives

Thaís A. S. Oliveira[a,†], Jackson B. A. Silva,[a,†] Nagela B. S. Silva,[b,†] Paulo C. A. Félix,[c†] Andreia M. de Oliveira,[b] Carlos H. G. Martins,[b] Lizandra G. Magalhães and Antônio E. M. Crotti*[, a]

[a] Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazi, e-mail: millercrotti@ffclrp.usp.br

[b] Instituto de Ciências Biomédicas, Universidade Federal de Uberlândia, Uberlândia, MG ,Brazil

[c] Núcleo de Pesquisa em Ciências Exatas e Tecnológicas, Universidade de Franca, Franca, SP, Brazil

† authors who had the same contribution to this work

Abstract: We have synthesized twenty-three 1,4-dihydropyridine derivatives (1,4-DHPs) by using a microwave-assisted one-pot multicomponent Hantzsch reaction and evaluated their antibacterial activity against a representative panel of cariogenic bacteria and their *in vitro* antileishmanial activity against *Leishmania* (*L.*) *amazonensis* promastigotes. Thirteen compounds were moderately active against *Streptococcus sanguinis*, *Streptococcus mitis*, and *Lactobacillus paracasei*. Compound **22** (diethyl 4-(3-methoxy-4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate) displayed moderate antibacterial activity against *S. mitis* and *S. sanguinis*, with a Minimum Inhibitory Concentration (MIC) of 500 µg/mL); compounds **8** (diethyl 4-(3-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate) and **10** (diethyl 4-(3-nitrophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate) were moderately active against *S. sanguinis* (MIC = 500 µg/mL) and very active against *L. amazonensis* promastigotes (IC_{50} = 43.08 and 34.28 µM, respectively). Among the eight 1,4-DHPs that were active (IC_{50} < 50 µM) against *L. amazonensis* promastigotes, compound **13** (diethyl 4-(3,4,5-trimethoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate) gave the lowest IC_{50} (24.62 µM). On the basis of our results, asymmetric 1,4-DHPs derived from dimedone exhibit antileishmanial potential.

Keywords: 1,4-dihydropyridine • *Leishmania amazonensis* • microwave irradiation • oral pathogens • *Streptococcus mutans*

Introduction

Dental caries, a progressive disease, is a public health concern: approximately 2.3 billion people suffer from long-standing tooth decay worldwide.[1] Dental caries is influenced by multiple factors, is primarily caused by plaque accumulation, and is characterized by dental hard tissue destruction.[2] The disease initiates with salivary proteins adhering to the tooth surface, which triggers plaque formation. Then, bacteria attach to the biofilm, and the acids produced by them lead to demineralization, culminating in caries.[3] *Streptococcus mutans*, one of the primary bacteria associated with dental caries, can attach to biofilm substrates and establish a strongly acidic microenvironment (pH below 5.0), thereby contributing to hard tooth apatite demineralization and the onset of tooth decay.[3] Removing biofilm by regularly brushing the teeth and flossing is considered the most effective approach to prevent caries and other periodontal diseases.[4] However, most people fail to prevent biofilm from building up through mechanical removal, so chemical mouth rinses are needed to inhibit bacterial growth.[5] Chlorhexidine (CHD), the most widely employed anticariogenic agent,[6] has numerous unwanted effects—it can stain the teeth, irritate the tongue, modify the taste, and cause sore mouth or throat and wheezing/shortness of breath.[7]

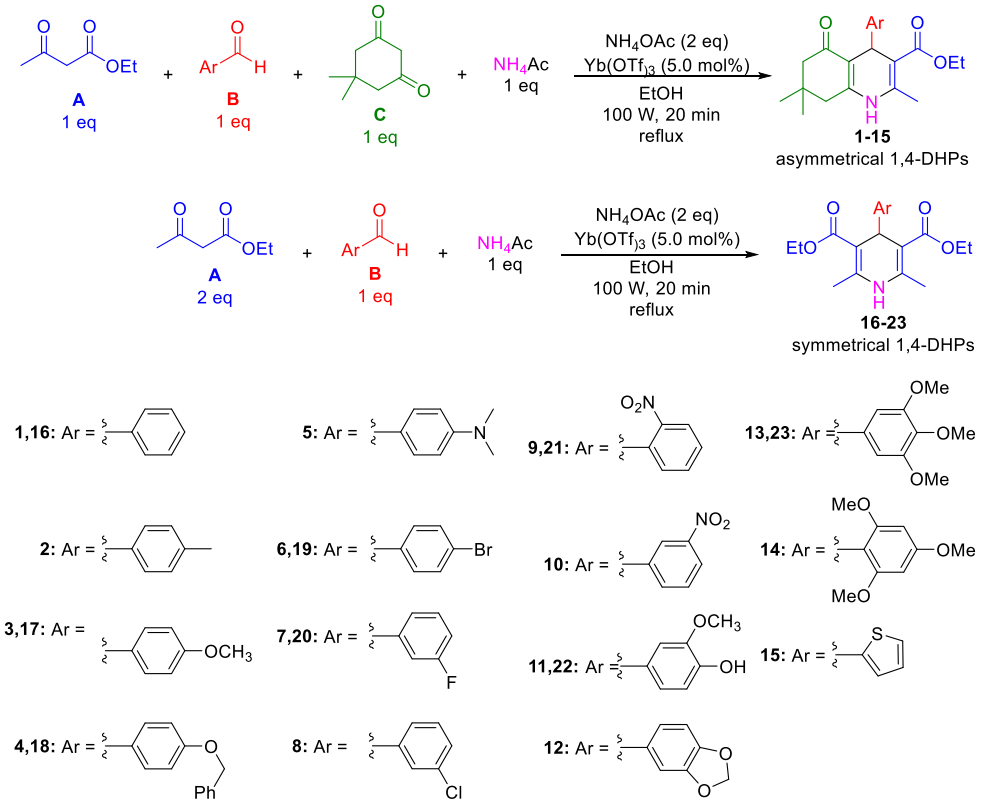
Leishmaniasis, a parasitic Neglected Tropical Disease (NTD) caused by protozoa of the genus *Leishmania*, is estimated to lead to about 30,000 deaths annually.[8] Depending on the infecting *Leishmania* species and the mammalian host's immunological and nutritional status, this disease can manifest in the tegumentary (TL) or visceral (VL) forms. *L. amazonensis* is the main causative agent of TL in the Americas.[9] TL can cause skin lesions that may either self-heal or progress to disfiguring scars and can extensively destroy nasopharyngeal mucosal tissues.[10] Diagnosing and treating TL early is difficult because the disease evolves slowly and covers large skin areas.[10] Moreover, the drugs that are available for treating leishmaniasis (e.g., pentavalent antimmonials, amphotericin B, pentamidine, miltefosine, and paramomycin) have disadvantages that include toxicity, high cost, and emergence of parasitic resistance.[11]

Several compounds with pharmacological activities, such as bronchodilating, anticonvulsant, hypertensive, and calcium channel blocking action, bear the 1,4-dihydropyridine nucleus (1,4-DHP).[12] These compounds are also known for their ability to reverse multi-drug resistance.[13]. In addition, several compounds of this class are active against bacteria [14-17] and parasites.[18-20]

As part of our interest in exploring the antimicrobial and antiparasitic activities of natural[21-23] and synthetic[8, 24, 25] compounds, and on the basis of the previous reports on the antibacterial[17, 26-28] and antileishmanial [18-20, 29] activities of 1,4-DHP derivatives (1,4-DHPs), in this study we have evaluated the antibacterial action of 23 synthetic 1,4-DHPs against a representative panel of cariogenic bacteria and their antileishmanial activity against *L. amazonensis* promastigotes.

Results and Discussion

We synthesized compounds **1–23** by using a microwave-assisted one-pot Hantzsch multicomponent reaction between an aromatic aldehyde and a β-dicarbonyl compound (*i.e.*, dimedone for asymmetric compounds **1–15**, and ethyl acetoacetate for symmetric compounds **16–23**) in ethanol; ammonium acetate was the nitrogen source,[17] and ytterbium triflate was the catalyst [30] (Scheme 1). At a microwave reactor potency of 100 W, all the aromatic aldehyde was consumed within 20 min. We isolated compounds **1–23** by vacuum filtration and purified them by recrystallization, to obtain yields varying from 15 to 40%. The yields were relatively low because by-products, especially pyridines,[31] 1,2-dihydropyridines,[31] and acridine-1,8-diones,[32] emerged, as detected in the ethanol-soluble phase of the recrystallization process (data not shown).



Scheme 1. Synthesis of asymmetric (**1–16**) and symmetric (**17–23**) 1,4-DHPs via the Hantzsch multicomponent reaction.

We evaluated the antibacterial activity of compounds **1–14** in terms of their minimum inhibitory concentration (MIC) values as compared to CHD dihydrochloride, used as positive control. Most compounds displayed MIC between 500 and 2000 µg/mL against all the studied bacteria (Table 1). We obtained the lowest MIC for compounds **8**, **10**, and **14** against *S. sanguinis* (MIC = 500 µg/mL), compounds **1**, **9**, **11**, **16**, **17**, **18**, **19**, **20**, **22**, and **23** against *L. paracasei* (MIC = 500 µg/mL), and compound **22** against *S. mitis* (MIC = 500 µg/mL).

Table 1. Minimum Inhibitory Concentration (MIC, in µg/mL)) values of compounds **1–23** against cariogenic bacteria. Values between parenthesis are MIC values given in mM.

1,4-DHP	*S. mutans*	*S. mitis*	*S. salivarus*	*S. sanguinis*	*S. sobrinus*	*E. faecalis*	*L. paracasei*
1	2000 (5.90)	1000 (2.95)	2000 (5.90)	1000 (2.95)	2000 (5.90)	2000 (5.90)	500 (1.47)
2	2000 (5.66)	1000 (2.83)	2000 (5.66)	1000 (2.83)	1000 (2.83)	>2000 (>5.66)	1000 (2.83)
3	1000 (2.71)	1000 (2.71)	1000 (2.71)	1000 (2.71)	1000 (2.71)	>2000 (>5.42)	1000 (2.71)
4	2000 (4.49)	1000 (2.25)	2000 (4.49)	1000 (2.25)	2000 (4.49)	>2000 (>4.49)	1000 (2.25)
5	>2000 (>5.23)	1000 (2.62)	2000 (5.23)	1000 (2.62)	2000 (5.23)	>2000 (>5.23)	1000 (2.62)
6	2000 (4.80)	1000 (2.40)	2000 (4.80)	1000 (2.40)	2000 (4.80)	>2000 (>4.80)	1000 (2.40)
7	1000 (2.80)	1000 (2.80)	1000 (2.80)	1000 (2.80)	1000 (2.80)	2000 (5.60)	1000 (2.80)
8	2000 (5.36)	1000 (2.68)	2000 (5.36)	500 (1.34)	1000 (2.68)	>2000 (>5.36)	1000 (2.68)
9	2000 (5.21)	1000 (2.60)	2000 (5.21)	1000 (2.60)	2000 (5.21)	2000 (5.21)	500 (1.30)
10	2000 (5.21)	1000 (2.60)	2000 (5.21)	500 (1.30)	1000 (2.60)	2000 (5.21)	1000 (2.60)
11	2000 (5.19)	1000 (2.60)	2000 (5.19)	1000 (2.60)	1000 (2.60)	2000 (5.19)	500 (1.30)
12	2000 (5.22)	1000 (2.61)	1000 (2.61)	2000 (5.22)	1000 (2.61)	>2000 (>5.22)	1000 (2.61)
13	2000 (4.66)	1000 (2.33)	2000 (4.66)	1000 (2.33)	2000 (4.66)	2000 (4.66)	1000 (2.33)
14	2000 (4.66)	1000 (2.33)	2000 (4.66)	500 (1.16)	1000 (2.33)	2000 (4.66)	1000 (2.33)
15	2000 (5.80)	1000 (2.90)	1000 (2.90)	1000 (2.90)	2000 (5.80)	2000 (5.80)	2000 (5.80)
16	2000 (6.08)	1000 (3.04)	1000 (3.04)	1000 (3.04)	2000 (6.08)	2000 (6.08)	500 (1.52)
17	2000 (5.57)	1000 (2.79)	1000 (2.79)	1000 (2.79)	2000 (5.57)	2000 (5.57)	500 (1.39)
18	2000 (4.60)	1000 (2.30)	1000 (2.30)	1000 (2.30)	2000 (4.60)	2000 (4.60)	500 (1.15)
19	2000 (4.91)	1000 (2.46)	1000 (2.46)	1000 (2.46)	2000 (4.91)	2000 (4.91)	500 (1.23)
20	2000 (5.76)	1000 (2.88)	1000 (2.88)	1000 (2.88)	2000 (5.76)	2000 (5.76)	500 (1.44)
21	2000 (5.35)	1000 (2.67)	1000 (2.67)	1000 (2.67)	2000 (5.35)	2000 (5.35)	1000 (2.67)
22	1000 (2.67)	500 (1.33)	1000 (2.67)	1000 (2.67)	2000 (5.33)	2000 (5.33)	500 (1.33)
23	2000 (4.77)	1000 (2.39)	1000 (2.39)	1000 (2.39)	2000 (4.77)	2000 (4.77)	500 (1.19)
CHD	0.92 (1.82[a])	3.68 (7.28[a])	0.92 (1.82[a])	1.84 (3.64[a])	0.92 (1.82[a])	3.68 (7.28[a])	1.84 (3.64[a])

CHD: chlorhexidine (positive control). [a] Value given in µM.

Regarding the *in vitro* antileishmanial activity of compounds **1–23** against *L. amazonensis* promastigotes (Table 2), only compounds **2**, **3**, **5**, **7**, **8**, **9**, **10**, **12**, **13**, **14**, and **15** inhibited motility by more than 60%. We obtained the lowest IC_{50} (half-maximum inhibitory concentration) values for compounds **13** (24.62 µM), **15** (33.84 µM), **2** (33.96 µM), **10** (34.29 µM), and **5** (35.18 µM). Compounds **8** (43.08 µM), **9** (47.65 µM), and **7** (49.55 µM) also gave IC_{50} lower than 50 µM. Amphotericin B (positive control), tested at 1.56 µM, inhibited 100% motility and has IC_{50} of 0.26 µM.

Studies have investigated the antibacterial potential of 1,4-DHPs, [14-16] but most have outlined the antibacterial activity of more complex 1,4-DHPs. For instance, Harikrishna and co-workers synthesized 17 pyrazole-containing 1,4-DHPs and obtained MIC ranging from 3.12 to 12.5 µg/mL against *Mycobacterium tuberculosis* and between 7.8 and 15.6 µg/mL against *Mycobacterium smegmatis*, *Staphylococcus aureus*,

Table 2. *In vitro* antileishmanial activity of compounds **1–23** against *L. amazonensis* promastigotes after treatment for 24 h.

Compound	% motility inhibition ± S.D.	IC_{50} (μM)[b]
1	0 ± 10.00	n.d.
2	73.86 ± 8.60	33.96 (29.44 – 38.48)
3	61.82 ± 4.93	>50
4	54.50 ± 11.02	n.d.
5	64.52 ± 4.61	35.18 (29.48 – 43.46)
6	51.68 ± 6.53	n.d.
7	65.08 ± 3.37	49.55 (46.01 – 54.00)
8	72.40 ± 8.12	43.08 (37.23 – 51.15)
9	69.59 ± 8.98	47.65 (46.39 – 49.02)
10	61.70 ± 6.24	34.29 (31.27 - 37.84)
11	57.20 ± 3.82	n.d.
12	74.65 ± 7.80	>50
13	73.30 ± 9.27	24.62 (20.51 – 30.29)
14	65.08 ± 3.44	>50
15	73.60 ± 5.51	33.84 (30.14 – 37.54)
16	0 ± 4.51	n.d.
17	21.41 ± 8.00	n.d.
18	19.78 ± 5.51	n.d.
19	0 ± 2.00	n.d.
20	56.37 ± 8.50	n.d.
21	31.50 ± 4.0	n.d.
22	25.69 ± 6.0	n.d.
23	14.68 ± 5.00	n.d.
Amp B[a]	100.00 ± 0.00	0.259 (0.211 – 0.319)

[a] Amphotericin B (positive control); [b] calculated only for compounds that provide inhibition percentage higher than 60%. n.d.: not determined.

and *Pseudomonas aeruginosa*.[33] Akbarzadeh and co-workers (2010) reported enhanced antibacterial action for cloxacillin 2-methylsulfonyl imidazolyl-1,4-dihydropyridine derivatives against methicillin-resistant *S. aureus*.[34] More recently, Gomha and co-workers used the agar diffusion well method to show

that some 1,4-DHPs-1,2,4-triazole hybrids inhibit bacteria more effectively than amphotericin B, ampicillin, and gentamicin.[35] Only a few studies have addressed the antibacterial activity of the more structurally simple. For example, Gonzáles and co-workers reported that compound **10** exhibits strong antibacterial activity against *Helicobacter pylori* clarithromycin-resistant strains.[15] On the other hand, Kumar and co-workers described low antimicrobial activity for compounds **16** and **17** against *S. aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, and *P. aeruginosa*.[28] A 2-aminobenzophenone derived from compound **22** has been reported to be weakly active against *S. aureus* and *E. coli*.[26] Nevertheless, to the best of our knowledge, the antibacterial activity of 1,4-DHPs against cariogenic bacteria has not been reported yet.

According to the literature, compounds with MIC lower than or equal to 50 µg/mL, between 51 and 100 µg/mL, between 101 and 500 µg/mL, and between 501 and 1000 µg/mL denote very strong, strong, moderate, and weak activity, respectively, whereas MIC higher than 1000 denotes inactivity.[36-38] On the basis of these criteria, all the compounds tested here were somehow active against at least one of the selected cariogenic bacteria. The activity of most compounds varied from moderate to weak. *S. sanguinis* and *L. paracasei* were the most sensitively affected by the tested compounds. Compounds **8**, **10**, and **14** displayed moderate activity against *S. sanguinis* (MIC = 500 µg/mL), whilst compounds **1**, **9**, **11**, **16**, **17**, **18**, **19**, **20**, **22**, and **23** were moderately active (MIC = 500 µg/mL) against *L. paracasei*. According to these results, asymmetric 1,4-DHPs (*i.e.*, compounds **8**, **10**, and **14**) were slightly more active against *L. paracasei*, whereas *S. sanguinis* was more sensitively inhibited by symmetric 1,4-DHPs (*i.e.*, compounds **9**, **11**, **16**, **17**, **18**, **19**, **20**, **22**, and **23**). Saad and co-workers have recently outlined these differences in the antibacterial activity of symmetric and asymmetric nitrile-containing 1,4-DHPs and reported different antibacterial action of these compounds against Gram-positive (*S. aureus* and *Bacillus subtilis*) and Gram-negative (*E. coli* and *P. aeruginosa*) bacteria.[29] Liang and co-workers have also reported that the antibacterial activity of symmetric and asymmetric compounds against cariogenic bacteria are different [24].

Our results on the antibacterial activity of 1,4-DHPs against cariogenic bacteria also indicated that the nature of the substituent at their benzene ring plays a key role in their antibacterial action. Although the compounds that displayed the lowest MIC had various substituents (*i.e.*, F, Cl, Br, NO_2, OH, OMe, and -OBn) at different positions of the aromatic ring, most of these compounds bore at least one methoxy group (-OCH_3) (*i.e.*, compounds **11**, **14**, **17**, **22**, and **23**). These results agreed with data previously reported by Vieira and co-workers, who stated that the presence of a methoxy group in the aromatic ring is essential for the antibacterial activity against cariogenic bacteria.[24]

While literature data on the antibacterial activity of 1,4-DHPs against cariogenic bacteria are scarce, the antileishmanial activity of this class of compounds has been extensively reported.[18-20, 29] In general,

compounds with IC_{50} lower than 10 μM, between 10 and 50 μM, between 50 and 100 μM, and higher than 100 μM are considered very active, active, moderately active, and inactive, respectively.[8] On the basis of these criteria, compounds **2**, **5**, **7**, **8**, **9**, **10**, **13**, and **15** were active against *L. amazonensis* promastigotes (Table 2). None of the symmetric 1,4-DHPs (*i.e.*, compounds **16–23**) inhibited the *L. amazonensis* promastigote motility significantly at the screened concentration (50 μM), in agreement with the findings of Pollo and co-workers, who showed that the symmetric compounds **22** and **23** were inactive against *L. amazonensis* amastigotes.[20]

Compound **13**, an asymmetric 1,4-DHP bearing methoxy groups at the aromatic ring positions 3, 4, and 5, provided the lowest IC_{50} (24.82 μM). Genestra and co-workers have previously discussed the methoxy group plays in antileishmanial and antitrypanosomal activities. The authors compared the action of several amidine derivatives against trypanosomatids, including *L. amazonensis* and *Trypanosoma cruzi*, and found that the most effective compound contains a methoxy group as a substituent.[39] The same research group demonstrated that the methoxy group lowers the number of *L. amazonensis* promastigotes pre-treated with the methoxy compound by destroying the interiorized parasites without harming the host cell despite nitrite production.[40] However, only the presence of the methoxy group does not ensure antileishmanial activity, as evidenced by the inactivity of compounds **22** and **23**.

The halogenated compounds **7** (49.55 μM) and **8** (43.08 μM), bearing fluorine and chlorine atoms at aromatic ring position 3, respectively, presented similar IC_{50}. In contrast, the difference between the IC_{50} of nitro compounds **9** (a 2-NO_2) and **10** (a 3-NO_2) revealed that the position of the NO_2 group in the aromatic ring affected the antileishmanial action. The structure of compound **10** (IC_{50} = 34.29 μM) resembled the structure of nitrendipine, with *in vitro* antileishmanial activity against *L. amazonensis* promastigotes (IC_{50} = 38.32 ± 6.66 μM) reported by Reimão and co-workers.[19] The antileishmanial activity of these nitroaromatic compounds may be related to their ability to act as redox-active agents and to increase ROS (reactive oxygen species) generation in *Leishmania* parasites, to dissipate the mitochondrial potential.[41]

Amlopidine and lacipidine, two 1,4-DHPs known for their Ca^{2+} channel blocking properties, are used to treat hypertension.[42, 43] Palit and Ali evaluated whether it was feasible to use oral amlopidine and lacipidine to treat VL given that these compounds inhibit DHPs during *Leishmania donovani* infection *in vitro* and in infected BALB/c mice receiving the drug orally. These authors reported that amlopidine and lacipidine inhibit *L. donovani* promastigotes by inhibiting oxygen consumption in a dose-dependent manner, triggering caspase 3-like activation-mediated programmed cell death of the parasite.[18] In principle, amlopidine, lacipidine, and compounds **2**, **5**, **7**, **8**, **9**, **10**, **12**, **14**, and **15** have similar structures, so the mechanisms of the antileishmanial action of these compounds may be the same as mechanisms

reported by Palit and Ali for amlopidine and lacipidine. These structural similarities notwithstanding, the mode of action of compounds **2**, **5**, **7**, **8**, **9**, **10**, **12**, **14**, and **15** cannot be elucidated only on the basis of the data gathered herein.

Conclusions

Among the 1,4-DHPs tested herein, 13 were moderately active against *S. sanguinis*, *S. mitis*, and *L. paracasei*, while compound **22** was moderately active against *S. mitis* and *S. sanguinis* (MIC = 500 µg/mL), and compounds **8** and **10** were moderately active against *S. sanguinis* (MIC = 500 µg/mL) and very active against *L. amazonensis* promastigotes (IC_{50} = 43.08 and 34.28 µM, respectively). Compounds **2**, **5**, **7**, **9**, **13**, and **15** were also active against *L. amazonensis* promastigotes; compound **13** gave the lowest IC_{50} (24.62 µM). This is the first report on the antibacterial activity of 1,4-DHPs against cariogenic bacteria. Despite the literature studies on the antileishmanial activity of 1,4-DHPs, the results obtained here for asymmetric 1,4-DHPs not only contribute to knowledge about the antiparasitic activity of this class of compounds but also demonstrate that there is still room for exploring the potential action of new and already known 1,4-DHPs against different *Leishmania* species and forms of the parasite.

Experimental Section

Synthesis of 1,4-Dyhidropyridines Compounds

The 1,4-DHPs were synthesized according to the multicomponent one-pot methodology described in the literature, with some modifications.[30] In the general procedure, 2.0 mmol of dimedone (Aldrich), 2.0 mmol of ethyl acetoacetate (Aldrich), and 0.06 g (5.0 mol%) of ytterbium triflate (Aldrich), as reaction catalyst, were diluted in ethanol (5.0 mL). Subsequently, 2.0 mmol of benzaldehyde (Aldrich) and 2.0 mmol of ammonium acetate (Scientific Exodus) were added. All the reagents were added at room temperature. The reaction mixture was taken to the microwave reactor CEM FocusedMicrowave™ Synthesis System, model Discover (CEM Corp, Matthews, NC), set in the Power Time, where it was maintained for 20 min at a fixed power of 100 W. Compounds **1-23** were identified based on data from their NMR (^{1}H, ^{13}C, and DEPT 135) and mass spectra, as well on their comparison with the literature data.[30, 44-57] All the compounds were isolated and tested as mixtures of enantiomers.

Antibacterial Assays

The *in vitro* antibacterial activity of 1,4 DHPs was evaluated in terms of their minimum inhibitory concentration (MIC) values.[58] To this end, *S. mutans* (ATCC 25175), *S. mitis* (ATCC 49456), *S. salivarius* (ATCC 25975), *S. sanguinis* (ATCC 10556), *S. sobrinus* (ATCC 33478), *Enterococcus faecal*is (ATCC4082), and

L. casei (ATCC 11578) were assayed by the broth microdilution method, in 96-well microplates. The bacterial colonies were cultured in blood agar (Difco Labs, Detroit, MI, USA) at 37 °C for 24 h. Further standardization of the inoculum quantity was accomplished on a spectrophotometer Femto (São Paulo, Brazil) operating at a wavelength of 625 nm, to match 0.5 in the McFarland scale (1.5 x 10^8 CFU/mL). Compounds **1–23** were dissolved in dimethyl sulfoxide (DMSO; Merck, Darmstadt, Germany) and tryptic soy broth (TSB, Difco), to obtain final concentrations varying from 1.9 to 4000 µg/mL. Inoculated microplate wells containing DMSO (1%) and TSB (1:5 (v/v) and 100%) were employed as negative control. A non-inoculated well was also added, to ensure medium sterility. Chlorhexidine (CHD) dihydrochloride (Sigma–Aldrich, St. Louis) at concentrations ranging from 0.0115 to 5.9 µg/mL in TSB (Difco) was employed as positive control. The microplates were sealed with plastic film and incubated at 37 °C for 24 h. Next, 30 µL of revealing 0.02% resazurin (Sigma–Aldrich, St. Louis) was added to each microplate well, to indicate microbial viability.[58] Experiments were conducted in three replicates for each microorganism. MIC values were assessed by analysis of the compound capacity to prevent the color of the resazurin solution from changing.[59]

Antileishmanial Assays

The *L.* (*Leishmania*) *amazonensis* strain (MHOM/BR/PH8) was maintained *in vitro* in RPMI 1640 medium (Gibco) supplemented with 10% SBF (Cultilab – Campinas, Brazil), 10,000 U/mL penicillin (Cultilab, Campinas, Brazil), and 10,000 µg/mL streptomycin (Cultilab, Campinas, Brazil) at pH 7.4 and 25 ºC in a BOD (Biochemical Oxygen Demand) oven (Quimis®, Diadema, BR). The culture medium was changed every three days and from the sixth day of cultivation (beginning of the stationary phase); the promastigote forms were collected in the supernatant. Installation and maintenance of *L.* (*L.*) *amazonensis* parasites was approved on October 28, 2019 by the animal use committee of the University of Franca (CEUA no. 3830250919).

The cultures were maintained as described previously, and 1 x 10^6 promastigote forms of *L.* (*L.*) *amazonensis* were transferred into each well of a 96-well plate. Compounds **1–13** were previously diluted in DMS) (Synth, São Paulo, BR) and added to the wells of the 96-well plate (Kasvi, São José dos Pinhais, Brazil) at a concentration of 50 µM for initial screening. The plates were incubated in a BOD oven at 25 °C for 24 or 48 h, and the activity was determined by evaluating the inhibition of flagellar motility by counting in a Neubauer chamber (Glass, Porto Alegre, Brazil) under an optical microscope. The negative control was RPMI 1640 medium (Gibco) containing 0.1% DMSO (Synth, São Paulo, Brazil). Amphotericin B (Amp B) at 1 µM previously diluted in DMSO (Synth, São Paulo, Brazil) was used as positive control.

To determine the half-maximum inhibitory concentration (IC_{50}), compounds that inhibited flagellar motility by more than 60% were further evaluated at concentrations ranging from 1.56 to 50 μM; for analogs and Amp B (Eurofarma, São Paulo, Brazil) at concentrations varying from 0.19 to 3.12 μM was used as positive control. Results are expressed as the mean percentage of motility inhibition relative to the negative control (0.1% DMSO). Two independent experiments were performed in triplicate. IC_{50} was determined through non-linear regression curves by using GraphPad Prism software version 8.0 for Windows (GraphPad Software, San Diego California, USA).

Author Contributions

T.A.S.O. and J.B.A.S. synthesized and identified the compounds; N.B.S.S, P.C.A.F, and A.M.O performed the antibacterial and antileishmanial assays; C.H.G.M. and L.G.M. supervised the antibacterial and antileishmanial, respectively; A.E.M.C. designed and drafted the manuscript. All the authors have read the final manuscript and approved its submission.

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Conflict of Interests

The authors declare no conflict of interest.

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