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Article

Production of Highly Active Antiparasitic Compounds from the Controlled Halogenation of the *Arrabidaea brachypoda* Crude Plant Extract

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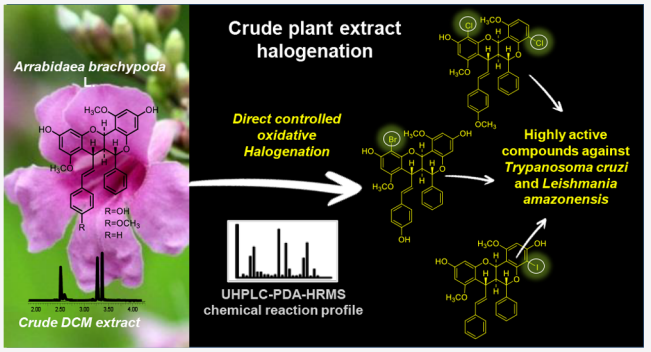
ABSTRACT: Direct halogenation of phenolic compounds present in the CH_2Cl_2 extract of the roots of *Arrabidaea brachypoda* was investigated to enhance chemodiversity. The approach is based on eco-friendly reactions using NaBr, NaI, and NaCl in aqueous media to generate multiple "unnatural" halogenated natural products from crude extracts. The halogenation reactions, monitored by UHPLC-PDA-ELSD-MS, were optimized to generate mono-, di-, or trihalogenated derivatives. To isolate these compounds, the reactions were scaled up and the halogenated analogues were isolated by semipreparative HPLC-UV and fully characterized by NMR and HR-MS data. All of the original 16 halogenated derivatives were evaluated for their antiparasitic activities against the parasites *Leishmania amazonensis* and *Trypanosoma cruzi*. Compounds presenting selective antiparasitic activities against one or both parasites with IC_{50} values comparable to the reference were identified.



The neglected tropical diseases represent a group of illnesses related to poverty and poor sanitation and are mainly present in tropical and subtropical countries. Around 20 illnesses, currently affecting a billion individuals, make up 12% of the total health burden in the world.[1] Among these diseases, leishmaniasis and Chagas disease are vectors transmitted via protozoan infections. Both infections suffer currently from limited chemotherapy and therefore are highly associated with huge social burdens, as well as the rise of morbidity and mortality. Thus, both diseases have a significant economic impact on the economy in developing countries.[2]

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, affects 6−7 million people and can lead to severe myocarditis and/or complications of the digestive tract.[3] Chagas disease was mainly confined to Latin America, but in the last decades, it has spread to other continents.[4] *T. cruzi* infection is curable if treatment with one of the two drugs available, nifurtimox and benznidazole, is initiated soon after infection.[5] However, these drugs are associated with severe toxicity and have low efficacy to cure patients with chronic Chagas disease.[6] Since none of the currently available anti-*T. cruzi* drugs are ideal, new treatments for Chagas disease are urgently needed.

Leishmaniasis is a group of diseases caused by protozoan parasites belonging to the *Leishmania* genus and transmitted by the bite of the female sand fly vector.[7] The diseases are endemic in 98 countries. Around 58 000 cases of visceral leishmaniasis and 220 000 cases of the cutaneous disease are officially reported annually worldwide.[8] The first-line treatment is performed with the pentavalent antimonials. Although still used, this chemotherapy presents several limitations, such as serious side effects, including patient death, a prolonged course of treatment, and the emergence of drug resistance. Second-line treatments, such as amphotericin B, pentamidine, and miltefosine, are also prescribed depending on the clinical manifestation and endemic zone. However, these treatments are again related to serious side effects resulting in high-cost therapy, low patient compliance, and treatment failure.[7,9]

From this perspective, it is more important than ever to explore new chemical entities to identify effective parasiticides that are effective against *T. cruzi* and *Leishmania* ssp. In this regard, natural products represent a source of unique and

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Table 1. Experimental Conditions for the Halogenation of the DCM Extract of *A. brachypoda*. Aliquots of Each Reaction (150 μL) Obtained between Time 0 and 48 h and Analyzed by UHPLC-PDA-MS

reaction	time (h) control	cosolvent	salt	H_2O_2	HOAc	temp (°C)
1	0, 1, 2, 3, 4, 6, 8, 24, 32, 48	MeCN:H_2O (6.5 mL:6.5 mL)	NaBr 1 equiv	21 equiv	5 mL	40
2	0, 1, 2, 3, 4, 6, 8, 24, 32, 48	MeCN: H_2O (6.5 mL:6.5 mL)	NaCl 1 equiv	21 equiv	5 mL	40
3	0, 1, 2, 3, 4, 6, 8, 24, 32, 48	MeCN: H_2O (6.5 mL:6.5 mL)	NaCl 10 equiv	21 equiv	5 mL	40
4	0, 1, 2, 3, 4, 6, 8, 24, 32, 48	MeCN: H_2O (6.5 mL:6.5 mL)	NaCl 100 equiv	21 equiv	5 mL	40
5	0, 1, 2, 3, 4, 6, 8, 24, 32, 48	MeCN: H_2O (6.5 mL:6.5 mL)	NaI 1 equiv	21 equiv	5 mL	40

[a]The number of equivalents was calculated based on the molecular mass average of three major compounds present in the DCM extract of *A. brachypoda*.

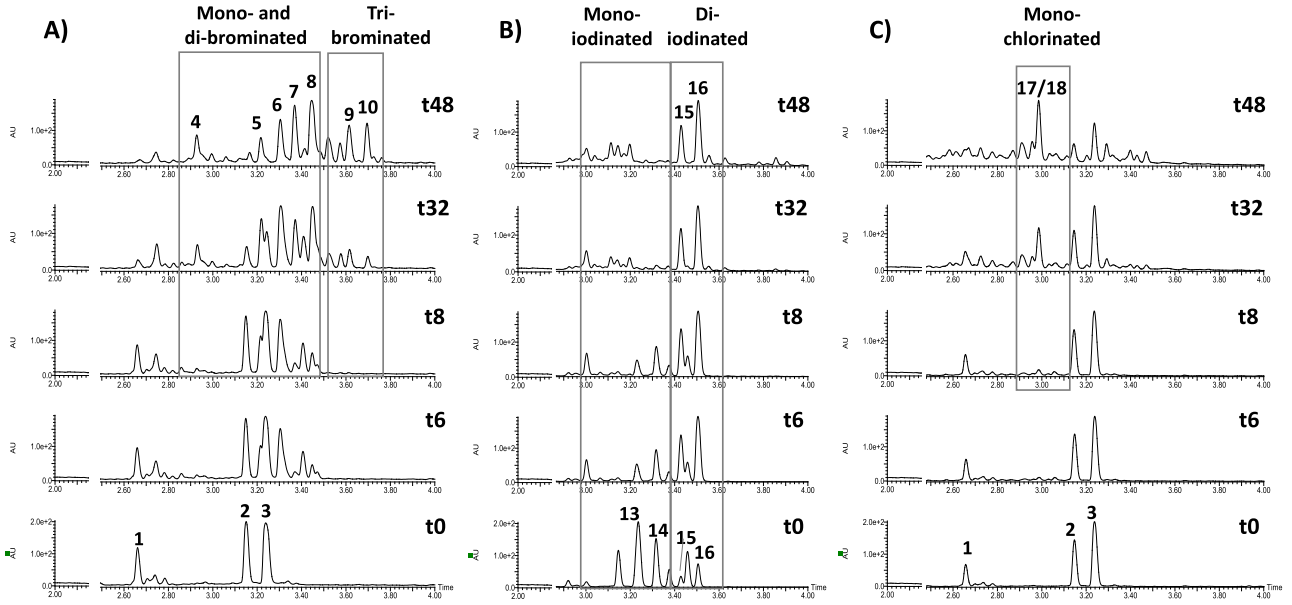

Figure 1. UHPLC-PDA analyses of the bromination of the DCM extract of *A. brachypoda* at different time points (0 to 48 h). (A) Reaction conditions: 1 equiv of NaBr, 21 equiv of H_2O_2, 5 mL of HOAc at 40 °C. (B) Reaction conditions: 1 equiv of NaI, 21 equiv of H_2O_2, 5 mL of HOAc at 40 °C. (C) Reaction conditions: 1 equiv of NaCl, 21 equiv of H_2O_2, 5 mL of HOAc at 40 °C.

diverse structural scaffolds. The discoveries of artemisinin and avermectin and their development into current medications are excellent examples of the potential of natural products as a source of antiparasitic compounds.[10,11]

Natural products are also an important source of active secondary metabolites against *T. cruzi* and *Leishmania* ssp.[11,12] In this context, a series of unusual biflavonoids have been isolated from the Brazilian medicinal plant *Arrabidaea brachypoda*, also known by the synonym name *Fridericia platyphylla* (Cham.) L.G. Lohmann.[13−15] These compounds showed interesting in vitro and in vivo activity against *T. cruzi*[13] and *Leishmania* ssp.[16] To further optimize the biological activities of this unique set of bioactive biflavonoids, the preparation of halogenated derivatives was considered. It is estimated that 20% of all low molecular weight active principles (API) in pharmaceutical drugs are halogenated.[17,18] The introduction of a carbon−halogen bond can have several effects such as the increase in thermal and oxidative stability, less sensitivity toward oxidation by liver P450 detoxification enzymes, and an increase in biological membrane permeability.[17,18]

Based on such considerations, a generic halogenation methodology recently developed in our laboratory[19] was applied directly to the crude plant extract of *Arrabidaea brachypoda* to generate halogenated derivatives from bioactive biflavonoids. The method has the advantage to produce NP derivatives with different levels of bromo, chloro, and iodo substitution. Reactions were monitored over time by UHPLC-PDA-ELSD-MS. Original and transformed extracts were also profiled by UHPLC-PDA-HRMS for rapid and accurate estimation of the halogenation level of all constituents. Finally, the resulting brominated compounds were isolated, fully characterized, and evaluated for their antiparasitic activities against *T. cruzi* and *L. amazonensis*.

RESULTS AND DISCUSSION

Before carrying out the halogenation reactions, the CH_2Cl_2 extract from the roots of *A. brachypoda* (DCM extract) was analyzed by HPLC-PDA to check the presence of the target bioactive biflavonoids. This confirmed the presence of three compounds with similar retention times and characteristic UV spectra corresponding to the data previously published (Figure S1, Supporting Information).[13,14]

The halogenation reactions were first carried out on a small scale in order to establish the optimal experimental conditions for production of halogenated compounds. The aim was to obtain ideally mono- or dihalogenated derivatives since in general polyhalogenated compounds have been described as toxic compounds.[20] Thus, the oxidative halogenation was performed with 50 mg of DCM extract of *A. brachypoda*, according to the conditions proposed by Bernini et al.[21] and recently optimized in our laboratory by Righi et al.[19] The

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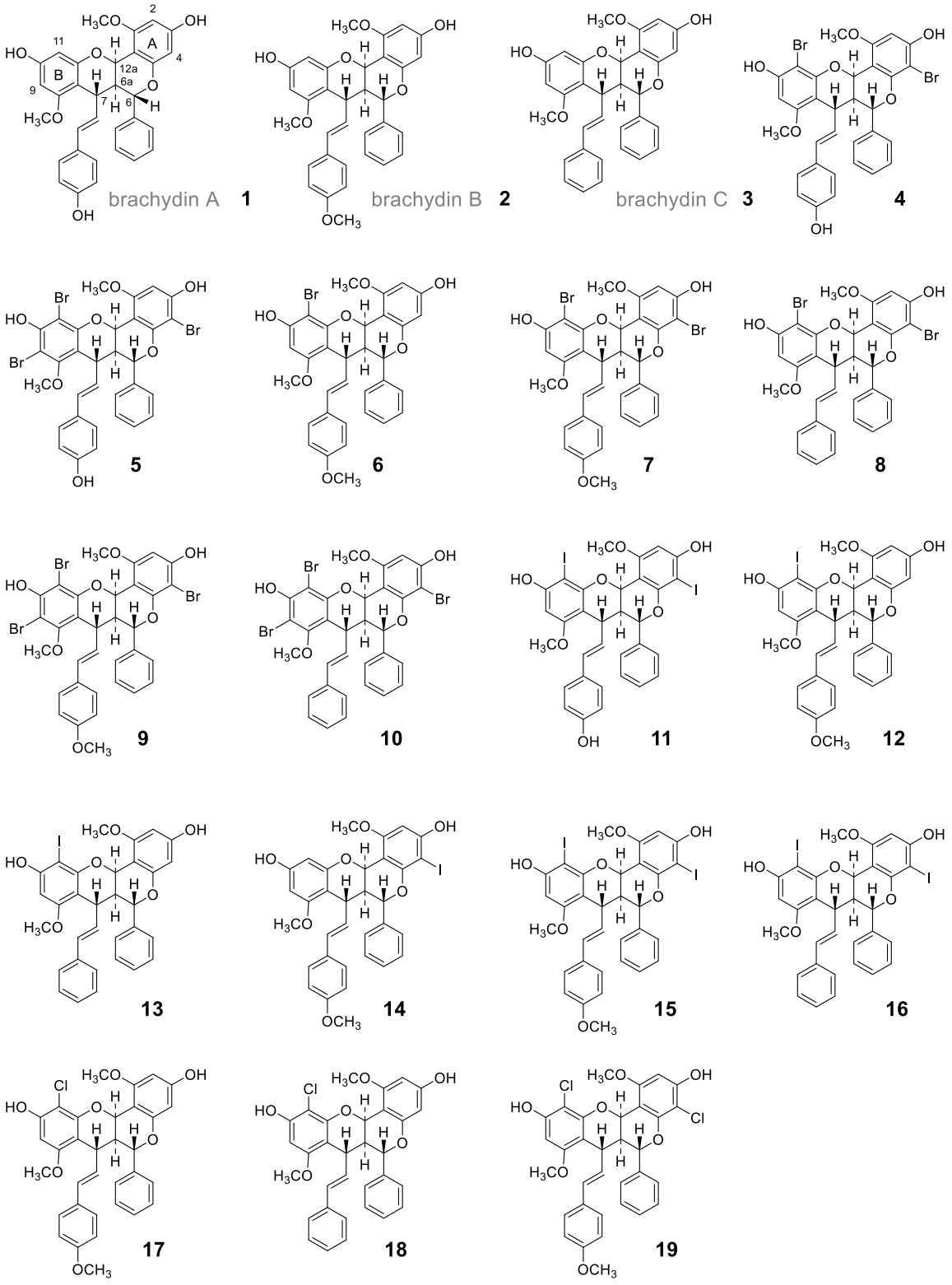

Figure 2. Structure of compounds **1**−**3** and the halogenated derivatives **4**−**19** obtained by the direct halogenation of the DCM extract of *A. brachypoda*.

bromination, chlorination, and iodination reactions were carried out by testing various experimental conditions summarized in Table 1. Owing to the high electronegativity and reactivity of fluorine, the fluorination process was not feasible via such a generic halogenation reaction. All reactions were monitored by UHPLC-PDA-MS analysis at 10 time points (0 to 48 h) (Figure 1). The number of equivalents was estimated assuming that the weight of the extract corresponded to the average of the molecular weight of the three major compounds brachydins A (**1**), B (**2**), and C (**3**) (MW 523).

In the case of the bromination reaction performed with 1 equiv of NaBr (reaction 1, Table 1) over 24 h, the monitoring

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by UHPLC-PDA-ELSD-MS revealed that the original compounds (**1**−**3**) started to disappear, affording mono-, di-, tri-, and tetrabrominated derivatives (**4**−**10**) (Figure 1A and Figure S2, Supporting Information). Based on these data, bromination reaction 1, with 1 equiv of NaBr, was selected for further studies.

For the chlorination reaction with 1 equiv of NaCl (reaction 2, Table 1), only a small amount of chlorinated derivatives was produced (Figure 1C). For this reason, the number of equivalents of NaCl was increased to 10 and 100 (reactions 3 and 4, Table 1). Using 100 equiv, the reaction finally occurred and a series of mono- and dichlorinated derivatives was produced in 24 h (Figure S3, Supporting Information).

The iodination reaction (reaction 5, Table 1) was successfully performed with 1 equiv of NaI. The UHPLC-PDA-ELSD-MS analyses at the different time points revealed that, due to the high reactivity of the iodine, the reaction generated all derivatives of interest after 1 h (Figure 1B).

After the various optimizations, three halogenation reactions were carried out with 500 mg of DCM extract (see Experimental Section). The reactions were monitored at the analytical scale by UHPLC-PDA-MS analyses at 10 time points (0 to 48 h). In general, the chemical profiles of all scaled-up reactions were similar to those at the analytical scale.

For the bromination reaction performed with 500 mg of DCM extract with 1 equiv of NaBr and 21 equiv of H_2O_2 (Table 1) for 48 h, the products were isolated after the transfer of analytical to semipreparative HPLC-UV conditions.[22,23] To ensure optimum chromatographic resolution, the mixture was injected into the semipreparative HPLC column using a dry load injection method recently developed in our laboratory (Figure S4, Supporting Information).[24] Using this approach, seven brominated derivatives were isolated in one step. The compounds were identified by spectroscopic methods as a monobrominated derivative (**6**), three dibrominated derivatives (**4**, **7**, and **8**), and three tribrominated derivatives (**5**, **9**, and **10**) (Figure 2).

The direct iodination reaction of the DCM extract (500 mg) was carried out with 1 equiv of NaI and 21 equiv of H_2O_2 (Table 1). As for the bromination reaction previously described, the reaction with NaI was stopped after 24 h, and this yielded three monoiodinated derivatives (**12**, **13**, and **14**) and three di-iodinated derivatives (**11**, **15**, and **16**) (Figure 2).

Finally, the direct chlorination of 500 mg of the DCM extract was performed with 100 equiv of NaCl and 21 equiv of H_2O_2 (Table 1). Like the bromination reaction, the reaction with NaCl was stopped after 24 h and yielded two monochlorinated derivative (**17** and **18**) and one dichlorinated derivative (**19**) (Figure 2).

The identification of the halogenated compounds was based on the NMR spectra of the starting compounds **1**−**3**.[13] The determination of the halogenation site(s) was done by the examination of the aromatic signal region between δ_H 5.8 and 6.4 of rings A and B. One pair of *meta*-coupled protons (J = 2.2 Hz) is typically observed for each of the two aromatic rings. The disappearance of a pair of doublets and the presence of a singlet indicate that a site has been halogenated on one of the two rings. The disappearance of two pairs of doublets and the presence of two singlets indicate a halogenation on each aromatic ring. The disappearance of a pair of doublets without the presence of a singlet is a sign that one of the two rings is dihalogenated. To locate the halogen substituent(s), HMBC correlations are essential. The H-2 proton is identified by its correlation with C-1, this oxygenated sp^2 carbon correlating with H-12a and MeO-1. Similarly, H-9 is identified by its correlation with C-8, itself correlating with H-7 and MeO-8. The H-4 and H-11 protons correlate with oxygenated sp^2 carbons that do not carry any methoxy group. The ROESY correlations between H-2 and MeO-1 and/or between H-9 and MeO-8 confirmed these assignments.

Derivatives **4**−**19** were subjected to a series of antiparasitic assays against the intracellular form of *Trypanosoma cruzi* and *Leishmania amazonensis*. The data acquisition was carried out on a high-content analysis system. First, the molecules were tested for cytotoxicity to mouse peritoneal macrophages. The cells were treated with different drug concentrations, and the cell viability was measured through resazurin (AlamarBlue) metabolism. None of the derivatives showed toxicity at the maximum concentration of 100 μM. The CC_{50} values of the compounds are shown as >100 μM, as shown in Tables 2 and 3.

Table 2. In Vitro Biological Assays of Compounds 4−19 against Amastigotes of *L. amazonensis*

compound	IC_{50} amastigotes (μM) (mean ± SEM)	CC_{50} macrophage (μM) (mean ± SEM)	SI (CC_{50}/IC_{50})
1[a]	>20	>20	ND
2[a]	2.2 ± 0.1	>20	9.1
3[a]	6.3 ± 1.3	>20	3.2
4	2.5 ± 0.8	>100	>40
5	6.5 ± 1.2	>100	>15
6	3.3 ± 0.8	>100	>30
7	3.6 ± 1	>100	>28
8	1.7 ± 0.2	>100	>59
9	4.5 ± 1	>100	>22
10	1.0 ± 0.3	>100	>100
11	7.8	>100	>13
13	>10	>100	ND
14	5.4 ± 0.4	>100	>19
15	4.7 ± 0.7	>100	>21
16	3.9 ± 0.4	>100	>26
17	>10	>100	N.D
18	1.2 ± 0.4	>100	>83
19	3.4 ± 0.9	>100	>29
amphotericin B[a]	0.2	>50	>217

[a]Data from our previous work.[16] [b]Reference compound. IC_{50} = inhibitory concentration to 50% of amastigotes; CC_{50} = cytotoxic concentration to 50% of macrophages; SI = selectivity index.

In the first screening against *L. amazonensis*, all compounds but **13** and **17** showed activity at 10 μM. The active molecules were reassayed to obtain the IC_{50} values, at concentrations of 10, 5, 2.5, and 1.25 μM. The automated image acquisitions and analysis were performed by the application of a previously standardized algorithm.[13] The IC_{50} values were estimated based upon two experiments performed in quadruplicate. These IC_{50} values are shown in Table 2.

We have previously described the anti-*Leishmanial* activity of compounds **1**−**3**.[16] For the majority of the compounds, the activity of the halogenated derivatives **4**−**19** was better than those of compounds **1**−**3**.[16] It is also interesting to highlight that bromination and iodination of the inactive natural compound **1** (IC_{50} > 20 μM)[16] generated active compounds **4**, **5**, and **11**. These results confirmed that the introduction of

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Table 3. In Vitro Biological Assays of Compounds 4−19 against Amastigote Forms of *T. cruzi*

compound	IC_{50} amastigotes (μM) (mean)	CC_{50} macrophage (μM) (mean)	SI (CC_{50}/IC_{50})
4	8.3	>100	>12
5	>10	>100	ND
6	3.6	>100	>28
7	4.7	>100	>21
8	1.6	>100	>63
9	3.8	>100	>26
10	2.9	>100	>34
11	1.8	>100	>56
13	1.9	>100	>53
14	1.6	>100	>63
15	2.5	>100	>40
16	6.5	>100	>15
17	1.4	>100	>71
18	1.4	>100	>71
19	2.2	>100	>45
benznidazole[a]	1.4 ± 0.2	>100	>35

[a]Reference compound. IC_{50} = inhibitory concentration to 50% of amastigotes; CC_{50} = cytotoxic concentration to 50% of macrophages; SI = selectivity index.

halogen substituents increases the biological activity of the original natural products and, in this case, also increased the selectivity toward the parasite, since no toxicity against the host cell was observed.

The best IC_{50} values against *L. amazonensis* amastigote forms were obtained with compounds **10** (IC_{50} 1.0 ± 0.3 μM) and **18** (IC_{50} 1.2 ± 0.4 μM), which also showed the highest selectivity index (above 100 and 83, respectively). These halogenated compounds had a better activity profile than the original compounds **2** and **3** against the same parasite (**2**: IC_{50} 2.2 ± 0.1 μM/SI 9.1 and **3**: IC_{50} 6.3 ± 1.3 μM/SI 3.2, respectively).[16]

The activity of compound **16** is illustrated in the micrographies presented in Figure S96 (Supporting Information). Noninfected macrophages have dot-free cytoplasm, suggesting the absence of amastigotes (Figure S96-A, Supporting Information). On the other hand, infected and nontreated groups show the presence of bright spots (amastigotes DNA) in the cytoplasm (Figure S96-B, Supporting Information). These spots are not observed in infected macrophages treated with compound **16** (Figure S96-C, Supporting Information), where empty vacuoles in the cytoplasm are observed.

All of the halogenated derivatives were also submitted to screening against the amastigote form of *Trypanosoma cruzi*. As for the assays against *L. amazonensis*, the majority of the compounds caused 100% inhibition at the concentration of 10 μM (Table 3). The only exception was the tribromo derivative **5**, suggesting that three atoms of bromine could interfere with the biological activity. It is noteworthy that **5** showed low activity against *L. amazonensis*. The activity of **18** against *T. cruzi* is highlighted in the micrography presented in Figure S97 (Supporting Information).

The best IC_{50} values against *T. cruzi* amastigote forms were obtained with compounds **8** (IC_{50} 1.6 μM), **14** (IC_{50} 1.6 μM), **17** (IC_{50} 1.4 μM), and **18** (IC_{50} 1.4 μM). All of these compounds showed elevated selectivity indices compared to the original compounds (>63 for **8** and **14**; >71 for **17** and **18**). Active compounds **4** and **11** were obtained from inactive natural product **1**,[11] confirming the hypothesis that the introduction of a certain number of halogen atoms can also increase the biological activity of the original natural products against *T. cruzi*, as well as its selectivity toward the parasite.

A procedure for a controlled halogenation reaction was successfully applied to the DCM extract of *A. brachypoda*. For this, an efficient monitoring was performed by a comprehensive UHPLC-PDA-ELSD-MS metabolite profiling, which allows an efficient localization of the halogenated compounds of interest directly in the crude reaction mixtures. Gradient transfer from the analytical HPLC to the semipreparative HPLC provided their effective isolation in one single step. This approach successfully generated 16 original halogenated derivatives, **4**−**19**.

All 16 halogenated derivatives were evaluated for their antiparasitic activities against the intracellular form of *L. amazonensis* and *T. cruzi*. The dibromo derivative **7** and monochloro derivative **18** showed remarkable biological activities against *L. amazonensis*, while compounds **8** (dibromo), **14** (monoiodo), **17** (monochloro), and **18** (dibromo) showed significant activities against *T. cruzi*. Most of the compounds showed selective activities against *T. cruzi* or *L. amazonensis*, except for **18**, which was active against both parasites, and **5**, which showed low activity against *L. amazonensis* and was inactive against *T. cruzi*.

The controlled halogenation process permitted the generation of new compounds that were highly selective against a parasitic target without any toxicity to the mammalian host cell. In the future, full synthesis of the most promising "non-natural" halogenated natural products will be carried out to conduct in vivo bioassays to evaluate pharmacological efficacy in experimental models of leishmaniasis and Chagas disease.

EXPERIMENTAL SECTION

General Experimental Procedures. NMR spectroscopic data were recorded on a Bruker Avance Neo 600 MHz NMR spectrometer equipped with a QCI 5 mm cryoprobe and a SampleJet automated sample changer (Bruker BioSpin, Rheinstetten, Germany). Chemical shifts are reported in parts per million (δ) using the residual CD_2Cl_2 (δ_H 5.32; δ_C 54.0) as internal standards for ^1H and ^{13}C NMR, respectively, and coupling constants (J) are reported in Hz. Complete assignments were obtained based on 2D NMR experiments: COSY, NOESY, HSQC, and HMBC. HRESIMS data were obtained on a Q-Exactive Focus mass spectrometer (Thermo Scientific, Bremen, Germany), using a heated electrospray ionization (HESI-II) source. Analytical HPLC was carried out on an HP 1260 system equipped with a photodiode array and an ELSD detector (Sedere, Alfortville, France). Semipreparative HPLC-UV analyses were conducted on a Shimadzu system (Kyoto, Japan) equipped with an LC-20A module pump, an SPD-20A UV/vis, a 7725I Rheodyne valve, and an FRC-10A fraction collector. The system is controlled by the software LabSolutions from Shimadzu.

Plant Material. The plant *A. brachypoda* was collected at Sant'Ana da Serra farm João Pinheiro, Minas Gerais, Brazil, in April 2010. At the ICEB of the José Badine Herbarium of the Federal University of Ouro Preto, Prof. M. C. T. B. Messias identified the plant and deposited a voucher specimen (No. 17935). The plant was collected in agreement with the Brazilian laws concerning the protection of biodiversity (SISGEN no. A451DE4).

Extraction and Isolation. The dried roots (300 g) were extracted successively by percolation, at room temperature, with 70% EtOH. The crude EtOH extract was evaporated to dryness to yield 11.8 g of dried material. Further liquid/liquid extractions were carried out using CH_2Cl_2 (1 L) and MeOH−H_2O (7:3) (1 L). The crude CH_2Cl_2 and MeOH fractions were obtained after decantation and evaporated to

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dryness under a vacuum at approximately 40 °C, yielding 4.44 g (37.7%) and 7.36 g (62.3%) of the dried fractions, respectively.

HPLC-PDA Analysis of the DCM Extract of *A. brachypoda*. HPLC-PDA-ELSD analyses were conducted on an HP 1260 system equipped with a photodiode array detector (Agilent Technologies, Santa Clara, CA, USA) connected to an ELSD Sedex 85 (Sedere, Oliver, France). The HPLC conditions were as follows: Interchim PF C_{18} HQ column (250 × 4.6 mm i.d., 10 μm, Moluçon, France); solvent system MeOH (B) and H_2O (A), both containing 0.1% formic acid, flow rate 1 mL/min, injection volume 20 μL, sample concentration 10 mg/mL in the mobile phase. The UV absorbance was measured at 254 and 280 nm, and the UV-PDA spectra were recorded between 190 and 600 nm. The ELSD detection parameters were as follows: pressure 3.5 bar, 40 °C, gain 8. This method was used for profiling the crude plant extract and to find the conditions for purifying the reactions.

General Procedure for the Plant Extract Halogenation at Analytical Scale. The DCM extract of *A. brachypoda* (50 mg) was solubilized in a solution of 6.5 mL of H_2O and 6.5 mL of MeCN. NaBr (1 equiv), NaI (1 equiv), or NaCl (1, 10, 100 equiv), and 5 mL of HOAc were added to the solution. Finally, H_2O_2 (21 equiv) was added to start the reaction. The mixture was stirred at 40 °C for 0 to 48 h. The reaction was monitored by UHPLC-PDA-ELSD-MS. The mixture was subsequently transferred to a funnel and extracted with EtOAc (3 × 10 mL). The organic phases were pooled, washed with H_2O (6 × 10 mL), and evaporated under reduced pressure, yielding the EtOAc fraction.

UHPLC-PDA-ELSD-MS of the Halogenation Reactions. The halogenation reactions were analyzed on a multidetection UHPLC-PDA-ELSD-MS platform fit on a QDa single quadrupole detector using the following conditions. Chromatographic separation was performed on a Waters Acquity UHPLC system interfaced with an Acquity QDa mass spectrometer (Waters), using a heated electrospray ionization source. Waters MassLynx 4.2 software was used for instrument control. Acquisition mode: full scan. The LC conditions were as follows: column Acquity UHPLC Waters BEH C_{18} (50 × 2.1 mm, 1.7 μm, Milford, MA, USA); mobile phase (A) H_2O with 0.1% formic acid; (B) MeCN with 0.1% formic acid. The elution followed a step gradient: 5% to 98% of B in 4.00 min, 98% to 98% in 0.80 min, 98% to 5% of B in 0.10 min, an equilibration at 5% of B during 1.10 min for the next injection; injection volume 4 μL; column manager temperature 40 °C; sample manager temperature 10 °C; and UV spectra PDA were recorded between 190 and 600 nm. ELSD detection was performed with a Buchi detector C 650 (Flawil, Switzerland) set at 45 °C, gain 8, and pressure 3.2 bar. The optimized ESI source parameters were as follows: source voltage, 0.8 kV (neg); source voltage, 1.2 kV (pos); heath gas flow rate (N_2), 7 bar; probe temperature, 600 °C; cone voltage, 15 V. Acquisition range: 150−1000 m/z in full-scan mode and sampling frequency 10 Hz.

Scale-up Bromination Reaction of the DCM Extract of *A. brachypoda*. The DCM extract of *A. brachypoda* (500 mg) was solubilized in a solution of 65 mL of H_2O and 65 mL of MeCN. NaBr (1 equiv) and 50 mL of HOAc were added to the solution. Finally, H_2O_2 (21 equiv) was added to start the reaction. The mixture was stirred at 40 °C for 0 to 48 h. The mixture was transferred to a funnel and extracted with EtOAc (3 × 100 mL). The organic phases were pooled, washed with H_2O (3 × 100 mL), dried over $MgSO_4$, and evaporated under reduced pressure, yielding the EtOAc fraction (707 mg). The halogenated derivatives were purified by semipreparative RP-HPLC using a PF C_{18} HQ Interchim column (250 × 21.2 mm i.d., 10 μm, Montluçon, France) equipped with a Waters C_{18} precolumn cartridge holder (20 × 4.6 mm i.d.). The flow rate was set at 21 mL/min and the UV detector at 280 nm. The injection was performed by a dry load according to the protocol recently developed in our laboratory.[24] The crude reaction mixture (40 mg) was mixed with 1.1 g of the Zeoprep C_{18} stationary phase. The mixture was conditioned in a dry-load cell (250 × 10 mm i.d.). The dry-load cell was connected to the Rheodyne valve.[24] Three injections were performed, and 120 mg of the crude reaction mixture was purified. The solvent system used was (A) H_2O and (B) MeCN, both containing 0.1% formic acid. The elution followed a step gradient: 55% isocratic of B for 45 min followed by 55% to 100% for 35 min. Fractions of 10 mL were collected containing the products of interest. The fractions were evaporated using a Genevac instrument. The content of each tube was transferred to an Eppendorf, yielding the brominated derivatives **4** (1.7 mg, 9%), **5** (0.8 mg, 4%), **6** (1.6 mg, 2%), **7** (2.9 mg, 2%), **8** (11.9 mg, 9%), **9** (1.9 mg, 1%), and **10** (2.7 mg 2%).

Scale-up Chlorination Reaction of the DCM Extract of *A. brachypoda*. The DCM extract of *A. brachypoda* (500 mg) was solubilized in a solution of 65 mL of H_2O and 65 mL of MeCN. NaCl (100 equiv) and 50 mL of HOAc were added to the solution. Finally, H_2O_2 (21 equiv) was added to start the reaction. The mixture was stirred at 40 °C for 0 to 24 h. The mixture was transferred to a funnel and extracted with EtOAc (3 × 100 mL). The organic phases were pooled, washed with H_2O (3 × 100 mL), dried over $MgSO_4$, and evaporated under reduced pressure, yielding the EtOAc fraction (800 mg). The halogenated derivatives were purified using the same conditions described above. Five injections were performed, and 200 mg of the crude reaction mixture was purified. The solvent system used was (A) H_2O and (B) MeOH, both containing 0.1% formic acid. The elution followed an isocratic mode of 71% (B) in 60 min yielding the chlorinated derivatives **17** (1.9 mg, 3%), **18** (3.9 mg, 6%), and **19** (4 mg, 7%).

Scale-up Iodination Reaction of the DCM Extract of *A. brachypoda*. The DCM extract of *A. brachypoda* (500 mg) was solubilized in a solution of 65 mL of water and 65 mL of MeCN. NaI (1 equiv) and 50 mL of HOAc were added to the solution. Finally, H_2O_2 (21 equiv) was added to start the reaction. The mixture was stirred at 40 °C for 0 to 48 h. The mixture was transferred to a funnel and extracted with EtOAc (3 × 100 mL). The organic phases were pooled, washed with H_2O (3 × 100 mL), dried over $MgSO_4$, and evaporated under reduced pressure, yielding the EtOAc fraction (830 mg). The halogenated derivatives were purified using the same conditions described above. Eight injections were performed, and 160 mg of the crude reaction mixture was purified. The solvent system used was (A) H_2O and (B) MeCN, both containing 0.1% formic acid. The elution followed an isocratic mode of 55% B in 55 min, yielding the iodinated derivatives: **11** (1.3 mg, 22%), **12** (1.2 mg, 4%), **13** (1.7 mg, 5%) **14** (1.6 mg, 5%), **15** (4.5 mg, 12%), and **16** (6.5 mg, 15%).

UHPLC-ESI-HRMS Analysis of the Pure Compounds. Chromatographic separation was performed on a Waters Acquity UHPLC system interfaced to a Q-Exactive Focus mass spectrometer (Thermo Scientific, Bremen, Germany), using a heated electrospray ionization (HESI-II) source. Thermo Scientific Xcalibur 3.1 software was used for instrument control. The LC conditions were as follows: column, Waters BEH C_{18} (50 × 2.1 mm, 1.7 μm, Milford, MA, USA); mobile phase, (A) H_2O with 0.1% formic acid; (B) MeCN with 0.1% formic acid; flow rate, 600 μL min^{-1}; injection volume, 2 μL; gradient, a linear gradient of 5−100% B over 7 min and isocratic at 100% B for 1 min. The optimized HESI-II parameters were as follows: source voltage, 2.5 kV (neg); source voltage, 3.5 kV (pos); sheath gas flow rate (N_2), 55 units; auxiliary gas flow rate, 15 units; spare gas flow rate, 3.0; capillary temperature, 275.00 °C (neg and pos), S-Lens RF Level, 45. The mass analyzer was calibrated using a mixture of caffeine, methionine-arginine-phenylalanine-alanine-acetate (MRFA), sodium dodecyl sulfate, sodium taurocholate, and Ultramark 1621 in a MeCN−MeOH−H_2O solution containing 1% formic acid by direct injection. The data-dependent MS/MS events were performed on the three most intense ions detected in full-scan MS (Top3 experiment). This method was used to obtain the high-resolution molecular mass for the compounds synthesized.

*4,11-Dibromobrachydin A (**4**):* amorphous beige powder; ^1H NMR (CD_2Cl_2, 600 MHz) δ 2.31 (1H, dt, J = 11.4, 2.5, 1.6 Hz, H-6a), 3.32 (1H, dt, J = 5.5, 1.6 Hz, H-7), 3.68 (3H, s, MeO-8), 3.88 (3H, s, MeO-1), 4.90 (1H, d, J = 11.4 Hz, H-6), 5.45 (1H, d, J = 2.5 Hz, H-12a), 5.92 (1H, dd, J = 15.8, 1.6 Hz, H-β), 6.00 (1H, dd, J = 15.8, 5.5 Hz, H-α), 6.31 (1H, s, H-9), 6.36 (1H, s, H-2), 6.71 (2H, d, J = 8.6 Hz, H-3″, H-5″), 7.14 (2H, d, J = 8.6 Hz, H-2″, H-6″), 7.27 (2H, m, H-2′, H-6′), 7.43 (3H, m, H-3′, H-4′, H-5′); ^{13}C NMR (CD_2Cl_2, 151 MHz) δ 33.5 (C-7), 41.3 (C-6a), 56.4 (MeO-8), 56.8

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(MeO-1), 64.0 (C-12a), 78.0 (C-6), 90.6 (C-4), 90.6 (C-11), 92.5 (C-9), 92.6 (C-2), 103.0 (C-7a), 104.7 (1 C-2b), 115.8 (C-3″, C-5″), 127.9 (C-2′, C-6′), 128.0 (C-2″, C-6″), 129.2 (C-3′, C-5′), 129.4 (C-4′), 130.0 (C-α), 130.4 (C-1″), 131.0 (C-β), 138.7 (C-1′), 150.2 (C-11a), 153.0 (C-4a), 153.5 (C-10), 155.3 (C-3), 155.7 (C-4″), 158.6 (C-8), 159.7 (C-1); HRESIMS m/z 681.0123 $[M + H]^+$ (calcd for $C_{32}H_{26}Br_2O_7$, 681.0118, Δppm = 0.73).

*4,9,11-Tribromobrachydin A (**5**):* amorphous beige powder; ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 2.42 (1H, dt, J = 11.5, 2.5, 1.6 Hz, H-6a), 3.37 (1H, dt, J = 6.0, 1.6 Hz, H-7), 3.55 (3H, s, MeO-8), 3.88 (3H, s, MeO-1), 4.88 (1H, d, J = 11.5 Hz, H-6), 5.48 (1H, d, J = 2.5 Hz, H-12a), 5.93 (1H, dd, J = 15.8, 1.6 Hz, H-β), 6.08 (1H, dd, J = 15.8, 6.0 Hz, H-α), 6.37 (1H, s, H-2), 6.72 (2H, d, J = 8.7 Hz, H-3″, H-5″), 7.16 (2H, d, J = 8.7 Hz, H-2″, H-6″), 7.34 (2H, m, H-2′, H-6′), 7.46 (3H, m, H-3′, H-4′, H-5′); ^{13}C NMR (CD_2Cl_2, 151 MHz) δ 34.8 (C-7), 41.1 (C-6a), 56.9 (MeO-1), 61.6 (MeO-8), 64.3 (C-12a), 78.0 (C-6), 90.5 (C-4), 92.7 (C-2), 104.3 (C-12b), 109.1 (C-7a), 115.9 (C-3″, C-5″), 128.2 (C-2″, 6″), 128.3 (C-2′, 6′), 129.4 (C-3′, C-5′), 129.9 (C-4′), 129.9 (C-1″), 130.0 (C-β), 131.9 (C-α), 138.2 (C-1′), 150.2 (C-11a), 150.7 (C-10), 153.0 (C-4a), 155.5 (C-3), 156.0 (C-4″), 156.2 (C-8), 159.7 (C-1); HRESIMS m/z 756.9088 $[M - H]^-$ (calcd for $C_{32}H_{25}Br_3O_7$, 756.9078, Δppm = 1.37).

*11-Bromobrachydin B (**6**):* amorphous, beige powder; ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 2.34 (1H, dt, J = 11.3, 2.5, 1.6 Hz, H-6a), 3.28 (1H, dt, J = 5.6, 1.6 Hz, H-7), 3.66 (3H, s, MeO-8), 3.75 (3H, s, MeO-4″), 3.86 (3H, s, MeO-1), 4.80 (1H, d, J = 11.3 Hz, H-6), 5.42 (1H, d, J = 2.5 Hz, H-12a), 5.92 (1H, dd, J = 15.8, 1.5 Hz, H-β), 6.02 (1H, d, J = 2.3 Hz, H-4), 6.02 (1H, dd, J = 15.8, 5.6 Hz, H-α), 6.12 (1H, d, J = 2.3 Hz, H-2), 6.30 (1H, s, H-9), 6.78 (2H, d, J = 8.8 Hz, H-3″, H-5″), 7.18 (2H, d, J = 8.8 Hz, H-2″, H-6″), 7.25 (2H, m, H-2′, H-6′), 7.40 (3H, m, H-3′, H-4′, H-5′); ^{13}C NMR (CD_2Cl_2, 151 MHz) δ 33.6 (C-7), 41.3 (C-6a), 55.8 (MeO-4″), 56.3 (MeO-8), 56.7 (MeO-1), 64.0 (C-12a), 77.2 (C-6), 90.7 (C-11), 92.3 (C-9), 92.7 (C-2), 96.2 (C-4), 103.2 (C-7a), 103.8 (C-12b), 114.4 (C-3″, C-5″), 127.8 (C-2″, C-6″), 127.9 (C-2′, C-6′), 129.1 (C-3′, C-5′), 129.3 (C-4′), 130.2 (C-α), 130.4 (C-1″), 130.8 (C-β), 139.2 (C-1′), 150.4 (C-11a), 153.4 (C-10), 157.2 (C-4a), 158.6 (C-8), 158.9 (C-3), 159.6 (C-4″), 160.8 (C-1); HRESIMS m/z 617.1167 $[M + H]^+$ (calcd for $C_{33}H_{29}BrO_7$ 617.1169, Δppm = −0.39).

*4,11-Dibromobrachydin B (**7**):* amorphous, beige powder; ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 2.31 (1H, dt, J = 11.4, 2.5, 1.6 Hz, H-6a), 3.32 (1H, dt, J = 5.5, 1.6 Hz, H-7), 3.68 (3H, s, MeO-8), 3.75 (3H, s, MeO-4″), 3.88 (3H, s, MeO-1), 4.90 (1H, d, J = 11.4 Hz, H-6), 5.45 (1H, d, J = 2.5 Hz, H-12a), 5.93 (1H, dd, J = 15.8, 1.6 Hz, H-β), 6.01 (1H, dd, J = 15.8, 5.5 Hz, H-α), 6.32 (1H, s, H-9), 6.36 (1H, s, H-2), 6.78 (2H, d, J = 8.8 Hz, H-3″, H-5″), 7.18 (2H, d, J = 8.7 Hz, H-2″, H-6″), 7.28 (2H, m, H-2′, H-6′), 7.43 (3H, m, H-3′, H-4′, H-5′); ^{13}C NMR (CD_2Cl_2, 151 MHz) δ 33.5 (C-7), 41.3 (C-6a), 55.8 (MeO-4″), 56.4 (MeO-8), 56.8 (MeO-1), 64.0 (C-12a), 78.1 (C-6), 90.6 (C-4), 90.6 (C-11), 92.5 (C-9), 92.6 (C-2), 103.1 (C-7a), 104.7 (C-12b), 114.4 (C-3″, C-5″), 127.8 (C-2″, C-6″), 127.9 (C-2′, C-6′), 129.2 (C-3′, C-5′), 129.4 (C-4′), 130.0 (C-α), 130.3 (C-1″), 131.0 (C-β), 138.7 (C-1′), 150.2 (C-11a), 153.0 (C-4a), 153.5 (C-10), 155.3 (C-3), 158.6 (C-8), 159.7 (C-1, C-4″); HRESIMS m/z 695.0285 $[M + H]^+$ (calcd for $C_{33}H_{28}Br_2O_7$ 695.0275, Δppm = 1.51).

*4,11-Dibromobrachydin C (**8**):* amorphous, beige powder; ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 2.33 (1H, ddd, J = 11.3, 2.5, 1.6 Hz, H-6a), 3.35 (1H, dt, J = 5.6, 1.6 Hz, H-7), 3.69 (3H, s, MeO-8), 3.87 (3H, s, MeO-1), 4.92 (1H, d, J = 11.3 Hz, H-6), 5.45 (1H, d, J = 2.5 Hz, H-12a), 5.71 (1H, s, OH), 5.83 (1H, s, OH), 6.00 (1H, dd, J = 15.8, 1.6 Hz, H-β), 6.16 (1H, dd, J = 15.8, 5.6 Hz, H-α), 6.32 (1H, s, H-9), 6.36 (1H, s, H-2), 7.16 (1H, m, H-4″), 7.24 (4H, m, H-2″, H-3″, H-5″, H-6″), 7.29 (2H, m, H-2′, H-6′), 7.44 (3H, m, H-3′, H-4′, H-5′); ^{13}C NMR (CD_2Cl_2, 151 MHz) δ 33.6 (C-7), 41.2 (C-6a), 56.4 (MeO-8), 56.8 (MeO-1), 64.0 (C-12a), 78.0 (C-6), 90.6 (C-4), 90.7 (C-11), 92.5 (C-9), 92.6 (C-2), 102.8 (7a), 104.7 (12b), 126.7 (C-2″, C-6″), 127.8 (C-4″), 127.9 (C-2′, C-6′), 129.0 (C-3″, C-5″), 129.2 (C-3′, C-5′), 129.4 (C-4′), 131.7 (C-β), 132.2 (C-α), 137.6 (C-1″), 138.6 (C-1′), 150.2 (C-11a), 153.0 (C-4a), 153.5 (C-10), 155.3 (C-3), 158.6 (C-8), 159.7 (C-1); HRESIMS m/z 665.0168 $[M + H]^+$ (calcd for $C_{32}H_{26}Br_2O_6$ 665.0169, Δppm = −0.13).

*4,9,11-Tribromobrachydin B (**9**):* amorphous, beige powder; ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 2.42 (1H, ddd, J = 11.4, 2.5, 1.6 Hz, H-6a), 3.38 (1H, dt, J = 6.0, 1.6 Hz, H-7), 3.55 (3H, s, MeO-8), 3.75 (3H, s, MeO-4″), 3.88 (3H, s, MeO-1), 4.88 (1H, d, J = 11.4 Hz, H-6), 5.49 (1H, d, J = 2.5 Hz, H-12a), 5.94 (1H, dd, J = 15.8, 1.6 Hz, H-β), 6.10 (1H, dd, J = 15.8, 6.0 Hz, H-α), 6.37 (1H, s, H-2), 6.79 (2H, d, J = 8.8 Hz, H-3″, H-5″), 7.21 (2H, d, J = 8.7 Hz, H-2″, H-6″), 7.34 (2H, m, H-2′, H-6′), 7.46 (3H, m, H-3′, H-4′, H-5′); ^{13}C NMR (CD_2Cl_2, 151 MHz) δ 34.9 (C-7), 41.1 (C-6a), 55.8 (MeO-4″), 56.9 (MeO-1), 61.6 (MeO-8), 64.3 (C-12a), 78.0 (C-6), 90.5 (C-4), 92.7 (C-2), 104.3 (C-12b), 109.2 (C-7a), 114.4 (C-3″, C-5″), 127.9 (C-2″, C-6″), 128.3 (C-2′, C- 6′), 129.4 (C-3′, C-5′), 129.8 (C-1″), 129.9 (C-4′), 130.0 (C-α), 132.0 (C-β), 138.2 (C-1′), 150.2 (C-11a), 150.6 (C-10), 153.0 (C-4a), 155.5 (C-3), 156.2 (C-8), 159.7 (C-1), 159.9 (C-4″); HRESIMS m/z 772.9291 $[M + H]^+$ (calcd for $C_{33}H_{27}Br_3O_7$ 772.9380, Δppm = −11.51).

*4,9,11-Tribromobrachydin C (**10**):* amorphous, beige powder; ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 2.44 (1H, ddd, J = 11.4, 2.5, 1.6 Hz, H-6a), 3.40 (1H, dt, J = 6.0, 1.6 Hz, H-7), 3.56 (3H, s, MeO-8), 3.88 (3H, s, MeO-1), 4.89 (1H, d, J = 11.4 Hz, H-6), 5.49 (1H, d, J = 2.5 Hz, H-12a), 6.01 (1H, dd, J = 15.8, 1.6 Hz, H-β), 6.25 (1H, dd, J = 15.8, 6.0 Hz, H-α), 6.37 (1H, s, H-2), 7.19 (1H, m, H-4″), 7.26 (4H, m, H-2″, H-3″, H-5″, H-6″), 7.35 (2H, m, H-2′, H-6′), 7.46 (3H, m, H-3′, H-4′, H-5′); ^{13}C NMR (CD_2Cl_2, 151 MHz) δ 34.9 (C-7), 41.0 (C-6a), 56.9 (MeO-1), 61.6 (MeO-8), 64.3 (C-12a), 78.0 (C-6), 90.5 (C-4), 92.7 (C-2), 94.7 (C-9 or C-11), 97.0 (C-9 or C-11), 104.3 (C-12b), 108.9 (C-7a), 126.8 (C-2″, C-6″), 128.1 (C-4″), 128.3 (C-2′, C-6′), 129.1 (C-3″, C-5″), 129.5 (C-3′, C-5′), 129.9 (C-4′), 132.3 (C-α), 132.6 (C-β), 137.1 (C-1″), 138.2 (C-1′), 150.2 (C-11a), 150.7 (C-10), 153.0 (C-4a), 155.5 (C-3), 156.2 (C-8), 159.7 (C-1). HRESIMS m/z 742.9246 $[M + H]^+$ (calcd for $C_{32}H_{25}Br_3O_6$ 742.9274, Δppm = −3.77).

*4,11-Iodobrachydin A (**11**):* amorphous, beige powder; ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 2.27 (1H, dt, J = 11.4, 2.5, 1.2 Hz, H-6a), 3.30 (1H, dt, J = 5.5, 1.2 Hz, H-7), 3.69 (3H, s, MeO-8), 3.89 (3H, s, MeO-1), 4.87 (1H, d, J = 11.4 Hz, H-6), 5.41 (1H, d, J = 2.5 Hz, H-12a), 5.90 (1H, dd, J = 15.8, 1.2 Hz, H-β), 5.98 (1H, dd, J = 15.8, 5.5 Hz, H-α), 6.32 (1H, s, H-9), 6.37 (1H, s, H-2), 6.68 (2H, d, J = 8.8 Hz, H-3″, H-5″), 7.11 (2H, d, J = 8.8 Hz, H-2″, H-6″), 7.28 (2H, m, H-2′, H-6′), 7.43 (3H, m, H-3′, H-4′, H-5′); ^{13}C NMR (CD_2Cl_2, 151 MHz) δ 33.4 (C-7), 41.9 (C-6a), 56.4 (MeO-8), 56.8 (MeO-1), 64.1 (C-12a), 66.0 (C-4), 66.4 (C-11), 78.5 (C-6), 92.0 (C-9), 92.1 (C-2), 102.6 (C-7a), 104.4 (C-12b), 116.0 (C-3″, C-5″), 127.9 (C-2′, C-6′), 128.0 (C-2″, C-6″), 129.1 (C-3′, C-5′, C-4′), 129.9 (C-α, C-1″), 130.9 (C-β), 138.7 (C-1′), 156.2 (C-4″), 156.3 (C-10), 158.1 (C-3), 160.0 (C-8), 161.2 (C-1); HRESIMS m/z 776.9833 $[M + H]^+$ (calcd for $C_{32}H_{26}I_2O_7$, 776.9840, Δppm = −1.00).

*11-Iodobrachydin B (**12**):* amorphous, beige powder; ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 2.34 (1H, dt, J = 11.3, 2.5, 1.6 Hz, H-6a), 3.25 (1H, dt, J = 5.7, 1.6 Hz, H-7), 3.66 (3H, s, MeO-8), 3.75 (3H, s, MeO-4″), 3.87 (3H, s, MeO-1), 4.77 (1H, d, J = 11.3 Hz, H-6), 5.41 (1H, d, J = 2.5 Hz, H-12a), 5.92 (1H, dd, J = 15.8, 1.6 Hz, H-β), 6.00 (1H, d, J = 2.3 Hz, H-4), 6.02 (1H, dd, J = 15.8, 5.7 Hz, H-α), 6.11 (1H, d, J = 2.3 Hz, H-2), 6.30 (1H, s, H-9), 6.77 (2H, d, J = 8.4 Hz, H-3″, H-5″), 7.18 (2H, d, J = 8.4 Hz, H-2″, H-6″), 7.25 (2H, m, H-2′, H-6′), 7.40 (3H, m, H-3′, H-4′, H-5′); ^{13}C NMR (CD_2Cl_2, 151 MHz) δ 33.7 (C-7), 41.6 (C-6a), 54.4, 55.7 (MeO-4″), 56.3 (MeO-8), 56.6 (MeO-1), 64.4 (C-12a), 66.6 (C-11), 77.2 (C-6), 91.9 (C-9), 92.8 (C-2), 96.0 (C-4), 102.8 (C-7a), 103.4 (C-12b), 114.3 (C-3″, C-5″), 127.8 (C-2″, C-6″), 128.0 (C-2′, C-6′), 129.1 (C-3′, C-5′), 129.2 (C-4′), 130.4 (C-α), 130.4 (C-1″), 130.6 (C-β), 139.2 (C-1′), 152.6 (C-11a), 156.1 (C-10), 157.1 (C-4a), 159.6 (C-3, C-4″), 160.1 (C-8), 160.8 (C-1); HRESIMS m/z 665.1044 $[M + H]^+$ (calcd for $C_{33}H_{29}IO_7$, 665.1031 Δppm = 1.99)

*11-Iodobrachydin C (**13**):* amorphous, beige powder; ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 2.36 (1H, dt, J = 11.2, 2.5, 1.6 Hz, H-6a), 3.28 (1H, dt, J = 5.7, 1.6 Hz, H-7), 3.67 (3H, s, MeO-8), 3.87 (3H, s, MeO-1), 4.78 (1H, d, J = 11.2 Hz, H-6), 5.41 (1H, d, J = 2.5 Hz, H-

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12a), 5.99 (1H, dd, J = 15.8, 1.6 Hz, H-β), 6.00 (2H, d, J = 2.3 Hz, H-4), 6.11 (1H, d, J = 2.3 Hz, H-2), 6.17 (1H, dd, J = 15.8, 5.7 Hz, H-α), 6.30 (1H, s, H-9), 7.16 (1H, m, H-4″), 7.25 (6H, m, H-2′, H-2″, H-3″, H-5″, H-6′, H-6″), 7.41 (3H, m, H-3′, H-4′, H-5′); ^{13}C NMR (CD_2Cl_2, 151 MHz) δ 33.8 (C-7), 41.5 (C-6a), 56.3 (MeO-8), 56.6 (MeO-1), 64.4 (C-12a), 66.8 (C-11), 77.2 (C-6), 91.9 (C-9), 92.8 (C-2), 96.0 (C-4), 102.6 (C-7a), 103.4 (C-12b), 126.7 (C-2″, C-6″), 127.7 (C-4″), 128.0 (C-2′, C-6′), 129.0 (C-3″, C-5″), 129.1 (C-3′, C-5′), 129.3 (C-4′), 131.3 (C-β), 132.6 (C-α), 137.7 (C-1″), 139.2 (C-1′), 152.6 (C-11a), 156.2 (C-10), 157.1 (C-4a), 159.6 (C-3), 160.1 (C-8), 160.8 (C-1); HRESIMS m/z 635.0929 $[M + H]^+$ (calcd for $C_{32}H_{27}IO_6$, 635.0925, Δppm = 0.61).

*4-Iodobrachydin B (**14**):* amorphous, beige powder; ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 2.22 (1H, dt, J = 11.3, 2.0, 1.6 Hz, H-6a), 3.27 (1H, dt, J = 5.6, 1.6 Hz, H-7), 3.68 (3H, s, MeO-8), 3.74 (3H, s, MeO-4″), 3.85 (3H, s, MeO-1), 4.96 (1H, d, J = 11.3 Hz, H-6), 5.32 (1H, overlapped, H-12a), 5.93 (1H, dd, J = 15.8, 1.5 Hz, H-β), 5.98 (1H, d, J = 2.3 Hz, H-11), 6.02 (1H, dd, J = 15.8, 5.5 Hz, H-α), 6.07 (1H, d, J = 2.3 Hz, H-9), 6.33 (1H, s, H-2), 6.77 (2H, d, J = 8.7 Hz, H-3″, H-5″), 7.18 (2H, d, J = 8.7 Hz, H-2″, H-6″), 7.28 (2H, td, J = 7.9, 2.9 Hz, H-2′, H-6′), 7.43 (3H, m, H-3′, H-4′, H-5′); ^{13}C NMR (CD_2Cl_2, 151 MHz) δ 33.1 (C-7), 41.7 (C-6a), 55.7 (MeO-4″), 56.2 (MeO-8), 56.5 (MeO-1), 62.6 (C-12a), 66.1 (C-4), 78.4 (C-6), 91.7 (C-2), 92.7 (C-9), 96.0 (C-11), 101.5 (C-7a), 104.6 (C-12b), 114.3 (C-3″, C-5″), 127.8 (C-2″, C-6″), 127.8 (C-2′, C-6′), 129.1 (C-3′, C-5′), 129.2 (C-4′), 130.5 (C-1″), 130.7 (C-α), 130.8 (C-β), 139.0 (C-1′), 154.4 (C-11a), 155.1 (C-4a), 157.6 (C-10), 158.0 (C-3), 159.6 (C-4″, C-8), 160.7 (C-1); HRESIMS m/z 665.1040 $[M + H]$+ (calcd for $C_{33}H_{29}IO_7$ 665.1031, Δppm = 1.39).

*4,11-Diodobrachydin B (**15**):* amorphous, beige powder; ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 2.29 (1H, dt, J = 11.4, 2.5, 1.5 Hz, H-6a), 3.32 (1H, dt, J = 5.6, 1.5 Hz, H-7), 3.70 (3H, s, MeO-8), 3.75 (3H, s, MeO-4″), 3.90 (3H, s, MeO-1), 4.88 (1H, d, J = 11.4 Hz, H-6), 5.42 (1H, d, J = 2.5 Hz, H-12a), 5.92 (1H, dd, J = 15.7, 1.5 Hz, H-β), 6.00 (1H, dd, J = 15.7, 5.6 Hz, H-α), 6.34 (1H, s, H-9), 6.40 (1H, s, H-2), 6.77 (2H, d, J = 8.6 Hz, H-3″, H-5″), 7.18 (2H, d, J = 8.6 Hz, H-2″, H-6″), 7.29 (2H, m, H-2′, H-6′), 7.43 (3H, m, H-3′, H-4′, H-5′); ^{13}C NMR (CD_2Cl_2, 151 MHz) δ 33.5 (C-7), 41.9 (C-6a), 55.8 (MeO-4″), 56.4 (MeO-8), 56.8 (MeO-1), 64.2 (C-12a), 66.5 (C-4), 66.9 (C-11), 78.3 (C-6), 92.0 (C-9), 92.1 (C-2), 102.7 (C-7a), 104.5 (C-12b), 114.4 (C-3″, C-5″), 127.8 (C-2″, C-6″), 127.8 (C-2′, C-6′), 129.1 (C-3′, C-5′), 129.3 (C-4′), 130.1 (C-α), 130.3 (C-1″), 130.9 (C-β), 138.7 (C-1′), 152.3 (C-11a), 155.1 (C-4a), 156.2 (C-10), 157.9 (C-3), 159.6 (C-4″), 160.1 (C-8), 161.2 (C-1); HRESIMS m/z 790.9990 $[M + H]^+$ (calcd for $C_{33}H_{28}I_2O_7$, 790.9997, Δppm = −0.92).

*4,11-Diodobrachydin C (**16**):* amorphous, beige powder; ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 2.31 (1H, ddd, J = 11.3, 2.5, 1.6 Hz, H-6a), 3.34 (1H, dt, J = 5.7, 1.6 Hz, H-7), 3.70 (3H, s, MeO-8), 3.89 (3H, s, MeO-1), 4.90 (1H, d, J = 11.3 Hz, H-6), 5.42 (1H, d, J = 2.5 Hz, H-12a), 5.53 (1H, s, 10OH), 5.65 (1H, s, 3OH), 5.99 (1H, dd, J = 15.8, 1.6 Hz, H-β), 6.16 (1H, dd, J = 15.8, 5.7 Hz, H-α), 6.35 (1H, s, H-9), 6.40 (1H, s, H-2), 7.16 (1H, m, H-4″), 7.24 (4H, m, H-2″, H-3″, H-5″, H-6″), 7.29 (2H, m, H-2′, H-6′), 7.44 (3H, m, H-3′, H-4′, H-5′); ^{13}C NMR (CD_2Cl_2, 151 MHz) δ 33.6 (C-7), 41.7 (C-6a), 56.4 (MeO-8), 56.8 (MeO-1), 64.2 (C-12a), 66.5 (C-4), 66.9 (C-11), 78.3 (C-6), 92.1 (C-9), 92.1 (C-2), 102.5 (C-7a), 104.5 (C-12b), 126.7 (C-2″, C-6″), 127.8 (C-4″), 127.8 (C-2′, C-6′), 129.0 (C-3″, C-5″), 129.2 (C-3′, C-5′), 129.3 (C-4′), 131.5 (C-β), 132.4 (C-α), 137.6 (C-1″), 138.7 (C-1′), 152.3 (C-11a), 155.1 (C-4a), 156.2 (C-10), 158.0 (C-3), 160.1 (C-8), 161.2 (C-1); HRESIMS m/z 760.9888 $[M + H]^+$ (calcd for $C_32H_{26}I_2O_6$, 760.9892, Δppm = −0.47).

*11-Chorobrachydin B (**17**):* amorphous, beige powder; ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 2.34 (1H, dt, J = 11.3, 2.3, 1.6 Hz, H-6a), 3.27 (1H, dt, J = 5.6, 1.6 Hz, H-7), 3.65 (3H, s, MeO-8), 3.75 (3H, s, MeO-4″), 3.85 (3H, s, MeO-1), 4.81 (1H, d, J = 11.3 Hz, H-6), 5.42 (1H, d, J = 2.3 Hz, H-12a), 5.92 (1H, dd, J = 15.8, 1.6 Hz, H-β), 6.00 (1H, d, J = 2.2 Hz, H-4), 6.02 (1H, dd, J = 15.8, 5.6 Hz, H-α), 6.10 (1H, d, J = 2.2 Hz, H-2), 6.26 (1H, s, H-9), 6.78 (2H, d, J = 8.8 Hz, H-3″, H-5″), 7.18 (2H, d, J = 8.8 Hz, H-2″, H-6″), 7.25 (2H, dd, J = 6.5, 3.0 Hz, H-2′, H-6′), 7.40 (3H, m, H-3′, H-4′, H-5′); ^{13}C NMR (CD_2Cl_2, 151 MHz) δ 33.6 (C-7), 41.2 (C-6a), 55.7 (MeO-4″), 56.3 (MeO-8), 56.6 (MeO-1), 63.9 (C-12a), 77.1 (C-6), 92.3 (C-9), 99.9 (C-11), 103.0 (C-7a), 103.2 (C-12b), 114.4 (C-3″, C-5″), 127.8 (C-2″, C-6″), 127.9 (C-2′, C-6′), 129.1 (C-3′, C-5′), 129.3 (C-4′), 130.2 (C-α), 130.4 (C-1″), 130.9 (C-β), 139.2 (C-1′), 149.7 (C-11a), 152.4 (C-10), 157.1 (C-4a), 157.6 (C-8), 159.6 (C-3, C-4″), 160.6 (C-1); HRESIMS m/z 573.1671 $[M + H]^+$ (calcd for $C_{33}H_{29}ClO_7$, 573.1675 Δppm = −0.62).

*11-Chlorobrachydin C (**18**):* amorphous, beige powder; ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 2.36 (1H, dt, J = 11.3, 2.4, 1.6 Hz, H-6a), 3.29 (1H, dt, J = 5.7, 1.6 Hz, H-7), 3.66 (3H, s, MeO-8), 3.85 (3H, s, MeO-1), 4.82 (1H, d, J = 11.3 Hz, H-6), 5.42 (1H, d, J = 2.4 Hz, H-12a), 5.99 (1H, dd, J = 15.8, 1.6 Hz, H-β), 6.00 (1H, d, J = 2.2 Hz, H-4), 6.10 (1H, d, J = 2.2 Hz, H-2), 6.18 (1H, dd, J = 15.8, 5.7 Hz, H-α), 6.26 (1H, s, H-9), 7.16 (1H, m, H-4′), 7.25 (6H, m, H-2′, H-2″, H-3″, H-5″, H-6′, H-6″), 7.41 (3H, m, H-3′, H-4″, H-5′); ^{13}C NMR (CD_2Cl_2, 151 MHz) δ 33.7 (C-7), 41.1 (C-6a), 56.3 (MeO-8), 56.6 (MeO-1), 63.9 (C-12a), 77.1 (C-6), 92.3 (C-9), 92.7 (C-2), 96.0 (C-4), 100.1 (C-11), 102.8 (C-7a), 103.1 (C-12b), 126.7 (C-2′, C-6′), 127.8 (C-4′), 127.9 (C-2″, C-6″), 129.0 (C-3″, C-5″), 129.1 (C-3′, C-5′), 129.3 (C-4″), 131.5 (C-β), 132.5 (C-α), 137.7 (C-1″), 139.2 (C-1′), 149.7 (C-11a), 152.5 (C-10), 157.1 (C-4a), 157.6 (C-8), 159.6 (C-3), 160.6 (C-1); HRESIMS m/z 543.1570 $[M + H]^+$ (calcd for $C_{33}H_{27}ClO_6$ 543.1569, Δppm = 0.20).

*4,11-Dichlorobrachydin B (**19**):* amorphous, beige powder; only proton analysis; ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 2.32 (1H, dt, J = 11.4, 2.4 Hz, H-6a), 3.31 (1H, overlapped, H-7), 3.67 (3H, s, MeO-8), 3.75 (4H, s, MeO-4″), 3.86 (3H, s, MeO-1), 4.90 (1H, d, J = 11.4 Hz, H-6), 5.45 (1H, d, J = 2.4 Hz, H-12a), 5.92 (1H, dd, J = 15.8, 1.3 Hz, H-β), 6.02 (1H, dd, J = 15.8, 5.5 Hz, H-α), 6.27 (1H, s, H-9), 6.32 (1H, s, H-2), 6.78 (2H, d, J = 8.8 Hz, H-3″, H-5″), 7.18 (2H, d, J = 8.8 Hz, H-2″, H-6″), 7.28 (2H, m, H-2′, H-6′), 7.42 (3H, m, H-3′, H-4′, H-5′); HRESIMS m/z 607.1282 $[M + H]^+$ (calcd for $C_{33}H_{28}Cl_2O_7$, 607.1285, Δppm = −0.47).

Host Cell Toxicity. Peritoneal exudate macrophages (5 × 10^5/mL) obtained from BALB/c mice after stimulation with thioglycolate (Sigma-Aldrich, St. Louis, MO, USA) for 5 days were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 0.2 U/mL of penicillin−streptomycin (complete DMEM) (all from Gibco/Life Technologies, Grand Island, NY, USA). The cells were seeded in 96-well plates, in 100 μL, and incubated overnight at 37 °C, 5% CO_2. The cells were treated with the halogenated compounds for 72 h of incubation time under the same conditions. The maximum concentration used to measure the toxicity was 100 μM, except amphotericin B, tested at the maximum concentration of 50 μM. Following treatments, cells were washed with sterile saline solution, and the cell viability was determined by the AlamarBlue assay (Thermo Fisher Scientific, Carlsbad, CA, USA), according to the manufacturer's instructions (10% of AlamarBlue in complete DMEM). Colorimetric readings were performed after 24 h at 570 and 600 nm, using a spectrophotometer. Cytotoxicity for 50% of the cells (CC_{50}) was determined after 72 h of treatment through a nonlinear regression curve fit. The percentage of cell decrease as compared to the nontreated control was determined. Calculations were performed using GraphPad Prism version 5.01.

In Vitro Macrophage Infection with *L. amazonensis* and *T. cruzi*. Peritoneal exudate macrophages (5 × 10^5/mL) obtained from BALB/c mice after stimulation with thioglycolate (Sigma-Aldrich) for 5 days were infected with *L. amazonensis* (MHOM/BR88/BA-125) or *T. cruzi* (Y strain) in 96-well plates. The cells were incubated for 24 h in complete DMEM at 37 °C with 5% of CO_2. For the *Leishmania* assay, the infection of the macrophages was realized with stationary phase promastigotes of *L. amazonensis* at a ratio of five parasites per host cell and incubated for 24 h at 35 °C, 5% CO_2. Those conditions allow the differentiation from promastigote to intracellular amastigote form (*L. amazonensis*). To remove the nonphagocytosed parasites, each well was washed with a sterile saline solution. For *T. cruzi* assay, macrophages were infected with trypomastigotes (10 parasites:1 host

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cell). Following 24 h of incubation at 37 °C, 5% CO_2, the noninternalized parasites were removed by washing with sterile saline solution. Those conditions allow the differentiation from trypomastigote to amastigote form (*T. cruzi*). The halogenated compounds were investigated at the maximum concentration of 10 μM, during 72 h. Amphotericin B was used as the reference compound for the assay against *L. amazonensis*, while benznidazole was used as the reference compound for the assays against *T. cruzi*.

Image Acquisition. After 72 h of incubation with the different compounds, the cells were fixed with a solution of paraformaldehyde 4% in phosphate-buffered saline during 20 min, at room temperature. For the *Leishmania* assay, cells were stained with Hoechst 33342 (Thermo Fischer Scientific) at 16 μM, while *T. cruzi*-infected cells were stained by the addition of 50 μL of a solution containing 4% paraformaldehyde and 4 μM Draq5 DNA dye (BioStatus, Shepshed, UK) per well. The images were acquired using the Operetta High-Content System (PerkinElmer, Waltham, MA, USA) in nonconfocal mode using a 20× air objective, for further segmentation and quantification. Five fields per well were analyzed for statistical reliability.

Automated Image Analysis. The images captured with the Operetta High-Content System were analyzed using Harmony software version 3.5.2 (PerkinElmer) through an algorithmic provided by the software building blocks. The Hoechst and Draq5 fluorescence allowed us to detect the nucleus (host cell and amastigote) and the cytoplasm of the host cell. Intracellular amastigotes were represented and detected as spots specifically in the cytoplasm region. The method was calibrated on a noninfected control to remove the background of the spots and choose the ones attributed to amastigote detection. Fluorescence-related parameters, such as median, mean, maximum intensity, and fluorescence contrast, were applied to automatically select the amastigotes on infected cells. Detection of amastigotes allowed the quantification of parasites. Detection of the nuclei allowed the quantification of cell numbers for quality control.

Calculation of Biological Response. The biological response was calculated based on the article published by Tegazzini et al. in 2016: % of response = $(X_1 - X_x/X_1 - X_2) \times 100$, where X_1 is the lowest activity (infected and untreated cells, maximum of parasite number), X_2 is the highest activity (noninfected cells), and X_x is the amastigote number after compound treatment at different concentrations.[25] The biological response was quantified with the total number of amastigotes. The IC_{50} was calculated using nonlinear regression. The percentage of cell decrease as compared to infected nontreated controls was determined. Calculations were performed using GraphPad Prism version 5.01.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00433.

Figures S1−S97 showing UHPLC-PDA-ELSD-MS analysis of the halogenation reactions, NMR spectra for the new compounds, and pictures of the biological activity of the new compounds (PDF)

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Notes

The authors declare no competing financial interest.

The raw data files for the UHPLC-PDA-ELSD-MS analysis of the halogenation reactions, NMR and HRMS data of the isolated compounds are available at the following link: DOI: 10.26037/yareta:wldglmsvs5ganjnjafjgabn6 mm.

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REFERENCES

(1) Mello, J. D. R. E.; Gomes, R. A.; Vital-Fujii, D. G.; Ferreira, G. M.; Trossini, G. H. G. *Chem. Biol. Drug Des.* **2017**, *90*, 1067−1078.
(2) WHO. *Neglected Tropical Diseases*; World Health Organization: Geneva, Switzerland, 2018.
(3) WHO. *Chagas Disease:Control and Elimination*; World Health Organization: Geneva, Switzerland, 2010.
(4) Angheben, A.; Gobbi, F.; Beltrame, A.; Marchese, V.; Requena-Mendez, A. *Trop. Med. Int. Health* **2015**, *20*, 246−246.
(5) Rodriques Coura, J.; de Castro, S. L. *Mem. Inst. Oswaldo Cruz* **2002**, *97*, 3−24.
(6) Morilla, M. J.; Romero, E. L. *Nanomedicine* **2015**, *10*, 465−481.
(7) WHO. *Research Priorities for Chagas Disease, Human African Trypanosomiasis and Leishmaniasis Technical Report of the TDR Disease Reference Group*; World Health Organization: Geneva, Switzerland, 2012.
(8) Alvar, J.; Velez, I. D.; Bern, C.; Herrero, M.; Desjeux, P.; Cano, J.; Jannin, J.; den Boer, M.; Team, W. H. O. L. C. *PLoS One* **2012**, *7*, No. e35671.
(9) Catta-Preta, C. M. C.; Mottram, J. C. *Nature* **2018**, *560*, 171−172.
(10) Hertweck, C. *Angew. Chem., Int. Ed.* **2015**, *54*, 14622−14624.
(11) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2016**, *79*, 629−661.
(12) Pink, R.; Hudson, A.; Mouries, M. A.; Bendig, M. *Nat. Rev. Drug Discovery* **2005**, *4*, 727−740.
(13) Da Rocha, C. Q.; Queiroz, E. F.; Meira, C. S.; Moreira, D. R.; Soares, M. B.; Marcourt, L.; Vilegas, W.; Wolfender, J. L. *J. Nat. Prod.* **2014**, *77*, 1345−1350.
(14) Da Rocha, C. Q.; de-Faria, F. M.; Marcourt, L.; Ebrahimi, S. N.; Kitano, B. T.; Ghilardi, A. F.; Luiz Ferreira, A.; De Almeida, A. C.; Dunder, R. J.; Souza-Brito, A. R.; Hamburger, M.; Vilegas, W.; Queiroz, E. F.; Wolfender, J. L. *Phytochemistry* **2017**, *135*, 93−105.
(15) Lohmann, L. G. Bignoniaceae in Lista de Espécies da Flora do Brasil. http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB112612 (accessed in May 27, 2020).
(16) Rocha, V. P. C.; Quintino da Rocha, C.; Ferreira Queiroz, E.; Marcourt, L.; Vilegas, W.; Grimaldi, G. B.; Furrer, P.; Allemann, E.; Wolfender, J. L.; Soares, M. B. P. *Molecules* **2019**, *24*, 1.
(17) Herrera-Rodriguez, L. N.; Meyer, H. P.; Robins, K. T.; Khan, F. *Chim. Oggi.* **2011**, *29*, 47−49.
(18) Herrera-Rodriguez, L. N.; Khan, F.; Robins, K. T.; Meyer, H. P. *Chim. Oggi.* **2011**, *29*, 31−33.
(19) Righi, D.; Marcourt, L.; Koval, A.; Ducret, V.; Pellissier, L.; Mainetti, A.; Katanaev, V. L.; Perron, K.; Wolfender, J. L.; Queiroz, E. F. *ACS Comb. Sci.* **2019**, *21*, 171−182.
(20) Coats, J. R. *Environ. Health Perspect.* **1990**, *87*, 255−262.
(21) Bernini, R.; Pasqualetti, M.; Provenzano, G.; Tempesta, S. *New J. Chem.* **2015**, *39*, 2980−2987.
(22) Guillarme, D.; Nguyen, D. T. T.; Rudaz, S.; Veuthey, J. L. *Eur. J. Pharm. Biopharm.* **2007**, *66*, 475−482.
(23) Guillarme, D.; Nguyen, D. T. T.; Rudaz, S.; Veuthey, J. L. *Eur. J. Pharm. Biopharm.* **2008**, *68*, 430−440.
(24) Queiroz, E. F.; Alfattani, A.; Afzan, A.; Marcourt, L.; Guillarme, D.; Wolfender, J. L. *J. Chromatogr. A* **2019**, *1598*, 85−91.
(25) Tegazzini, D.; Diaz, R.; Aguilar, F.; Pena, I.; Presa, J. L.; Yardley, V.; Martin, J. J.; Coteron, J. M.; Croft, S. L.; Cantizani, J. *Antimicrob. Agents Chemother.* **2016**, *60*, 3524−3532.

NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on September 9, 2020. Due to a production error, some of the chromatograms in Figure 1 appear in double. This has been corrected and the revised version was re-posted on September 11, 2020.

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