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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 tri-halogenated 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, totalize 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 reflecting 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 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.[13,14,15] 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 towards oxidation by liver P450 detoxification enzymes, and the 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 di-halogenated 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 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 hours) (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).

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

Reaction	Time (hours) control	Co-solvent	Salt	H_2O_2	HOAc	Temp.
1	0,1,2,3,4,6,8,24, 32,48	MeCN:H_2O (6.5 mL:6.5 mL)	NaBr 1 eq.	21 eq.	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 eq	21 eq.	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 eq	21 eq.	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 eq	21 eq.	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 eq	21 eq.	5 mL	40°

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

In the case of the bromination reaction performed with 1 equivalent of NaBr (reaction 1, Table 1) over 24 hours, the monitoring by UHPLC-PDA-ELSD-MS revealed that the original compounds (**1**-**3**) started to disappear affording mono-, di-, tri-, and tetra-brominated derivatives (**4**-**10**) (Figure 1A and Figure S2, Supporting Information). Based on these data, bromination reaction 1, with 1 equivalent of NaBr, was selected for further studies.

For the chlorination reaction with 1 equivalent 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

equivalents, the reaction finally occurred and a series of mono- and di-chlorinated derivatives was produced in 24 hours (Figure S3, Supporting Information).

The iodination reaction (reaction 5, Table 1) was successfully performed with 1 equivalent 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 hour (Figure 1B).

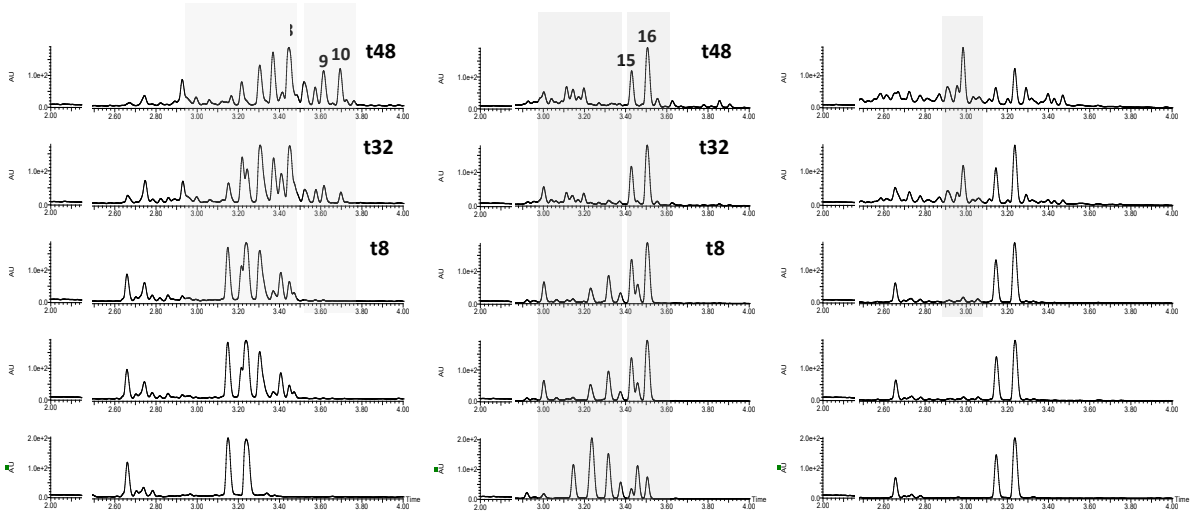

Figure 1. UHPLC-PDA analyses of the bromination of the DCM extract of *A. brachypoda* at different time points (0 to 48 hours). A) Reaction conditions: 1 eq. of NaBr, 21 eq. of H_2O_2, 5 mL of HOAc at 40°C. B) Reaction conditions: 1 eq. of NaI, 21 eq. of H_2O_2, 5 mL of HOAc at 40°C. C) Reaction conditions: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

After the various optimizations, three halogenation reactions were carried out with 500 mg 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 hours). In general, the chemical profiles of all up-scaled reactions were similar to those at the analytical scale.

For the bromination reaction performed with 500 mg of DCM extract with 1 eq. of NaBr and 21 eq. of H_2O_2 (Table 1) for 48 hours, the products were isolated after the transfer of analytical to semi-preparative HPLC-UV conditions.[22, 23] To ensure optimum chromatographic resolution,

the mixture was injected into the semi-preparative 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 mono-brominated derivative **6**, three di-brominated derivatives **4**, **7**, and **8** and three tri-brominated derivatives **5**, **9**, and **10** (Figure 2).

The direct iodination reaction of the DCM extract (500 mg) was carried out with 1 equivalent of NaI and 21 eq. of H_2O_2 (Table 1). As for the bromination reaction previously described, the reaction with NaI was stopped after 24 hours and this yielded three mono-iodinated 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 eq. of NaCl and 21 eq. of H_2O_2 (Table 1). Like the bromination reaction, the reaction with NaCl was stopped after 24 hours and yielded two mono-chlorinated derivative **17** and **18**, and one di-chlorinated derivative **19** (Figure 2).

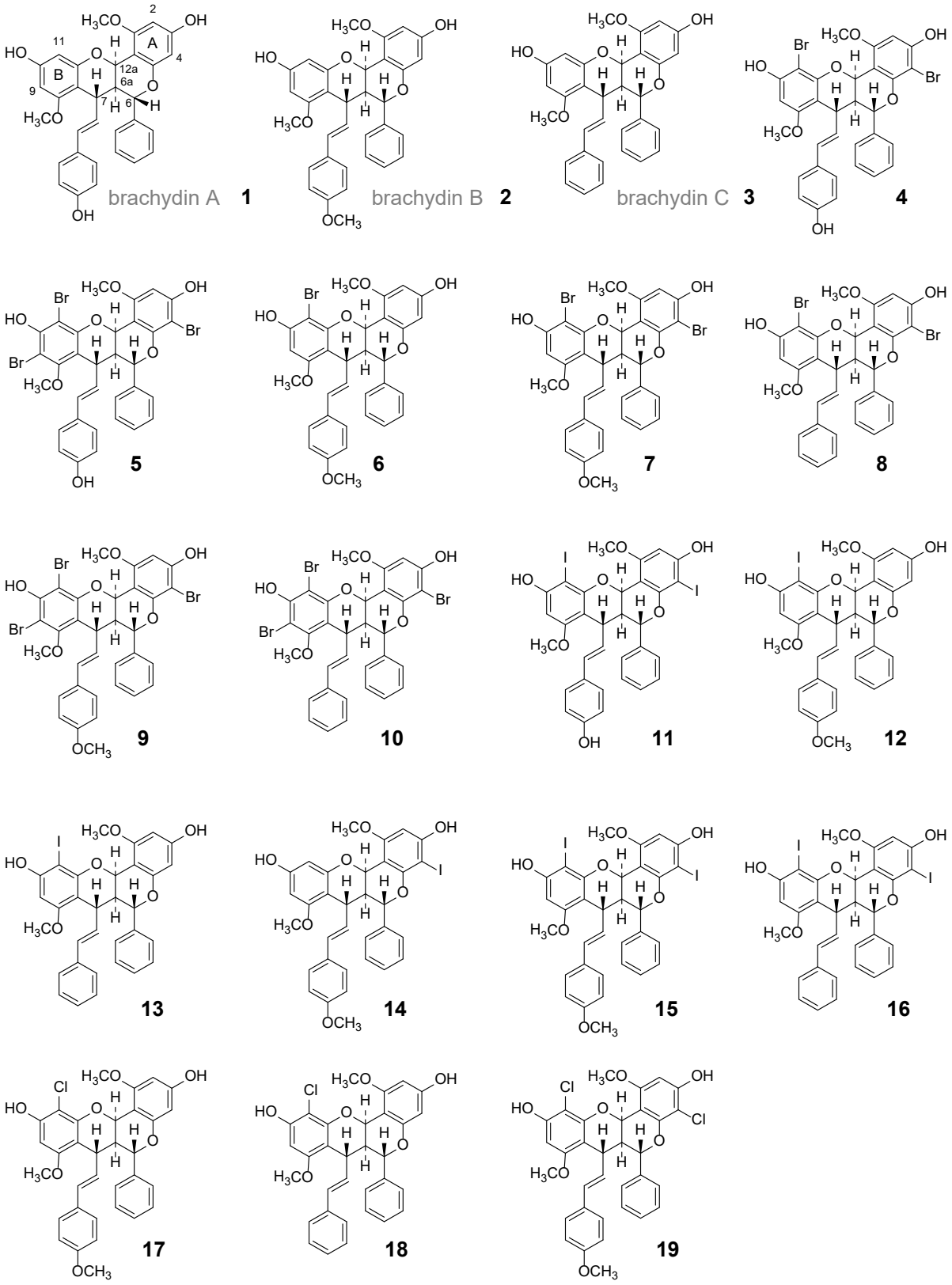

Figure 2. Structure of compounds **1**-**3** and the halogenated derivatives **4**-**19** obtained by the direct halogenation of the DCM extract of *A. brachypoda*.

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 indicates 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 di-halogenated. 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. 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 (Alamar blue®) 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.

In the first screening against *L. amazonensis*, all compounds but **13** and **17** showed activity at 10 μM. The active molecules were re-assayed 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.

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*	>20	>20	ND
2*	2.2 ± 0.1	>20	9.1
3*	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	N.D
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*	0.2	>50	>217

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

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 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} value 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). Non-infected macrophages have dot-free cytoplasm, suggesting the absence of amastigotes (Figure S96-A, Supporting Information). On the other hand, infected and non-treated 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 96) 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 Figure S97 (Supporting Information).

The best IC_{50} value 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 selective indices compared to the original compounds (>63 for **8** and **14**; >71 for **17** and **18**). Active compounds **4** and **11** were obtained from an 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.

Table 3. In vitro Biological Assays of Compounds **4-19** against Amastigote forms of *T. cruzi.*

Compounds	IC_{50} amastigotes (μM) (mean)	CC_{50} macrophage (μM) (mean)	SI (CC_{50}/IC_{50})
4	8.3	>100	>12
5	>10	>100	N.D
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*	1.4 ± 0.2	>100	>35

*Reference compound. IC_{50} = inhibitory concentration to 50% of amastigotes; CC_{50} = cytotoxic concentration to 50% of macrophages; SI = selective index

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 semi-preparative 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 actives 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 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). Semi-preparative HPLC-UV analyses were conducted on a Shimadzu system (Kyoto, Japan) equipped with an LC-20A module pumps, 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' Anada Serrafarm 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 n° A451DE4).

Extraction and Isolation. The dried roots (300 g) were extracted successively by percolation, at room temperature, with 70% EtOH. The crude EtOH extracts was evaporated yielding 11.8 g. 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 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 nm and 280 nm and the UV-PDA

spectra were recorded between 190 and 600 nm. The ELSD detection parameters were: 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 eq), NaI (1eq) or NaCl (1 eq, 10 eq, 100 eq), and 5 mL of HOAc was added to the solution. Finally, H_2O_2 (21 eq) was added to start the reaction. The mixture was stirred at 40°C for 0 to 48 hours. The reaction was monitored by UHPLC-PDA-ELSD-MS. The mixture was subsequently transferred to a funnel and extracted by with EtOAc (3 x 10 mL). The organic phases were pooled, washed with H_2O (6 x 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 single quadrupole detector QDa 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 equilibrated 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 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, 1.2kV (pos); heath

gas flow rate (N_2), 7 bar, probe temperature 600°C, cone voltage 15V. 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*. 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 eq) and 50 mL of HOAc were added to the solution. Finally, H_2O_2 (21 eq) was added to start the reaction. The mixture was stirred at 40°C for 0 to 48 hours. The mixture was transferred to a funnel and extracted with EtOAc (3 x 100 mL). The organic phases were pooled, washed with H_2O (3 x 100 mL), dried over $MgSO_4$ and evaporated under reduced pressure yielding the EtOAc fraction (707 mg). The halogenated derivatives were purified by semi-preparative RP-HPLC using an Interchim column PF C_{18} HQ (250 x 21.2 mm i.d., 10 µm, Montluçon, France) equipped with a Waters C_{18} pre-column cartridge holder (20 x 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 1.1g 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*. 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 eq) and 50 mL of HOAc was added to the solution. Finally, H_2O_2 (21 eq) was added

to start the reaction. The mixture was stirred at 40°C for 0 to 24 hours. The mixture was transferred to a funnel and extracted with EtOAc (3 x 100 mL). The organic phases were pooled, washed with H_2O (3 x 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*. DCM extract of *A. brachypoda* (500 mg) was solubilized in a solution of 65 mL of water and 65 mL of MeCN. NaI (1 eq) and 50 mL of HOAc were added to the solution. Finally, H_2O_2 (21 eq) was added to start the reaction. The mixture was stirred at 40°C for 0 to 48 hours. The mixture was transferred to a funnel and extracted with EtOAc (3 x 100 mL). The organic phases were pooled, washed with H_2O (3 x 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 were 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 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 $\mu L.min^{-1}$; 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.^{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), 3.88 (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 (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 $[M+H]^+$ (calculated for $C_{32}H_{26}Br_2O_7$, 681.0118, Δ ppm= 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 $[M-H]^-$ (calculated for $C_{32}H_{25}Br_3O_7$, 756.9078, Δ ppm= 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 $[M+H]^+$ (calculated for $C_{33}H_{29}BrO_7$ 617.1169, Δ ppm= -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 $[M+H]^+$ (calculated for $C_{33}H_{28}Br_2O_7$ 695.0275, Δ ppm= 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 $[M+H]^+$ (calculated for $C_{32}H_{26}Br_2O_6$ 665.0169, Δ ppm= -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 $[M+H]^+$ (calculated for $C_{33}H_{27}Br_3O_7$ 772.9380, Δ ppm= -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 $[M+H]^+$ (calculated for $C_{32}H_{25}Br_3O_6$ 742.9274, Δ ppm= -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 $[M+H]^+$ (calculated for $C_{32}H_{26}I_2O_7$, 776.9840, Δ ppm= -1.00).

*11-Iodobrachydin B (**12**):* Amorphous beige powder. ^1H 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 $[M+H]^+$ (calculated for $C_{33}H_{29}IO_7$, 665.1031 Δ ppm= 1.99)

*11-Iodobrachydin C (**13**):* Amorphous beige powder. ^1H 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-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 $[M+H]^+$ (calculated for $C_{32}H_{27}IO_6$, 635.0925, Δ ppm= 0.61).

*4-Iodobrachydin B (**14**):* Amorphous beige powder. ^1H 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]+ (calculated for $C_{33}H_{29}IO_7$ 665.1031, Δ ppm= 1.39).

*4,11-Diodobrachydin B (**15**):* Amorphous beige powder. ^1H 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]+ (calculated for $C_{33}H_{28}I_2O_7$, 790.9997, Δ ppm= -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 $[M+H]^+$ (calculated for $C_32H_{26}I_2O_6$, 760.9892, Δ ppm= -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 $[M+H]^+$ (calculated for $C_{33}H_{29}ClO_7$, 573.1675 Δ ppm= -0.62).

*11-Chlorobrachydin C (**18**):* Amorphous beige powder. ^1H 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 $[M+H]^+$ (calculated for $C_{33}H_{27}ClO_6$ 543.1569, Δ ppm= 0.20).

*4,11-Dichlorobrachydin B (**19**):* Amorphous beige powder. Only proton analysis. ^1H 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 $[M+H]^+$ (calculated for $C_{33}H_{28}Cl_2O_7$, 607.1285, Δ ppm= -0.47).

Host Cell Toxicity. Peritoneal exudate macrophages (5 x 10^5/mL) obtained from BALB/c mice after stimulation with thioglycollate (Sigma-Aldrich, St. Louis, MO, USA) for five 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 halogetated 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 Alamar blue 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 the 72 hours of treatment through a non-linear regression curve fit. The percentage of cell decrease as compared to the non-treated control. Calculations were performed using GraphPad Prism version 5.01.

In vitro Macrophage Infection with *L. amazonensis* and *T. cruzi*. Peritoneal exudate macrophages (5 x 10^5/mL) obtained from BALB/c mice after stimulation with thioglycolate (Sigma-Aldrich) for five 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 hours 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 hours at 35°C, 5% CO_2. Those conditions allow the differentiation from promastigote to intracellular amastigote form (*L. amazonensis*). To remove the non-phagocytosed parasites, each well was washed with a sterile saline solution. For *T. cruzi* assay, macrophages were infected with trypomastigotes (10 parasites:1 host cell). Following 24 hours of incubation at 37°C, 5% CO_2, the non-internalized 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 hours. 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 hours of incubation with the different compounds, the cells were fixed with a solution of paraformaldehyde 4% in phosphate buffered saline during 20 minutes, at room temperature. For the *Leishmania* assay, cells were stained with Hoechst 33342 (Thermo Fischer Scientific) at 16 µM while *T. cruzi*-infected cells 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 (Perkin Elmer, Waltham, MA, USA) in non-confocal mode using a 20X 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 the Harmony software version 3.5.2 (Perkin Elmer) 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 non-infected 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 = (X1-Xx/X1-X2) *100, where X_1 is the lowest activity (infected and untreated cells, maximum of parasite number), X_2 is the

highest activity (non-infected cells) and X_x being 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 non-linear regression. The percentage of cell decrease as compared to infected non-treated controls. Calculations were performed using GraphPad Prism version 5.01.

ASSOCIATED CONTENT

The Supporting Information is available free of charge. 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. 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:wldglmsvs5ganjnjafjgabn6mm.

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Notes

The authors declare no competing financial interest.

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