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Synthesis and *In Vitro* Evaluation of Tropane Halogenated-derivatives against Malaria, Sleeping Sickness, Chagas Disease and Leishmaniasis.

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Abstract:A series of twelve analogs carrying fluoro, chloro, bromo and iodo halogens on the ortho, meta and para positions of a benzoyloxytropane skeleton were synthesized by a simple acylation of 8-methyl-8-aza-bicyclo[3.2.1]octan-3α-ol by halogenobenzoyl chlorides. The compounds were evaluated *in vitro* against *Plasmodium falciparum (P. f.), Trypanosoma brucei brucei (T. b. b.), Trypanosoma cruzi (T. c.)* and *Leishmania infantum (L. i.)*. This study shows that the presence of a halogenated atom and its position on the aromatic ring are important for *in vitro* activity. Compounds **4** (IC_{50} = 3.6 µM), **8** (IC_{50} = 6.7 µM), **5** (IC_{50} = 8.1 µM) and **7** (IC_{50} = 9.5 µM) were found the most active against *P. f.*, whereas compounds **12** (IC_{50} = 5.1 µM), **11** (IC_{50} = 5.6 µM) and **9** (IC_{50} = 5.8 µM) exhibited the most pronounced activity against *T. b. b*. This series of compounds can be considered as non-toxic to the human cell line MRC-5.

Keywords: Antiplasmodial activity, antitrypanosomal activity, cytotoxicity, halogenobenzoyl derivatives, tropane derivatives, tropine acylation.

1. INTRODUCTION

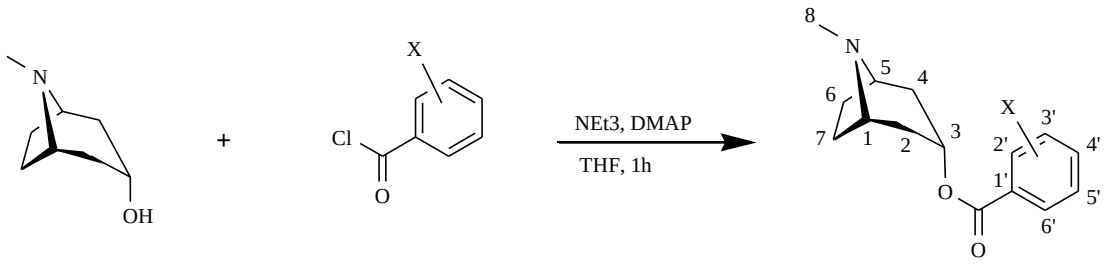
Diseases caused by protozoan parasites are responsible for considerable mortality and morbidity, particularly in tropical and subtropical areas throughout the world. The most prevalent is obviously malaria, although other common diseases such as South-American trypanosomiasis or Chagas disease and human African trypanosomiasis or sleeping sickness contribute to the overall burden of more than one billion people [1]. For example in malaria-endemic countries, the gross domestic product (GDP) is decreased by as much as 1.3% and the gross national product (GNP) up to 50% [2]. Hence, disease control by early diagnosis and treatment are crucial, but currently available drugs have several drawbacks including severe adverse reactions, toxicity, high cost, or are becoming less effective due to emergence of drug resistance [3]. Considering the compelling need for new and improved treatments, plants offer a vast reservoir of novel phytoconstituents and secondary metabolites as a valuable source of chemotherapeutic agents. For example, quinine from *Cinchona* species (Rubiaceae) and artemisinin from *Artemisia annua* (Asteraceae) are two plant-derived antimalarials [4] and have been used as a template for the development of semi-synthetic or synthetic drugs with an improved selectivity [5]. The utilization of natural products as templates in the synthesis of analogous compounds with enhanced potency plays an important role in the development of new drug candidates for innovative therapeutic approaches.

Tropane alkaloids are an important class of natural compounds with a prominent impact in medicine since ancient times. Hyoscyamine, its racemate atropine and scopolamine are very well known as anticholinergic drugs. Cocaine was used first as local anesthetic agent and is now known as a drug of abuse. In a previous study [6], we demonstrated that tropane alkaloids extracted from a Chilean plant *Schizanthus tricolor* Grau et Gronbach (Solanaceae) had antiplasmodial potential. As far as we are aware, there is no report on the antiparasitic activity of this class of compounds. It has been reported by Nogueira and Lopes [7] that brominated alkaloids demonstrated significant antiplasmodial activity against *Plasmodium falciparum*. Therefore, it appeared interesting to synthetize bromo-tropanes and other halogenated derivatives to evaluate their antiparasitic activity. In this follow-up study, a series of twelve halogenobenzoyloxy-tropane analogs (compounds **1**-**12**) and 3α-(benzoyloxy)-tropane **13** (Table **1**) were synthesized. They were tested not only against malaria (*Plasmodium falciparum* resistant to chloroquine and pyrimethamine), but also against other common parasitic protozoan diseases: sleeping sickness

(*Trypanosoma brucei brucei*), Chagas' disease (*Trypanosoma cruzi*) and leishmaniasis (*Leishmania infantum*). The structure-activity relationships (SARs) with regard to the nature and the position of the halogen atom on the aromatic ring will be discussed.

2. RESULTS AND DISCUSSION

The synthesis of the halogenobenzoyloxy-tropane was carried out by a simple acylation of 8-methyl-8-aza-bicyclo[3.2.1]octan-3α-ol (or α-tropine) by a halogenobenzoyl chloride with dimethylaminopyridine (DMAP) as catalyst (Scheme **1**).



Scheme 1. Acylation of α-tropine by a halogenobenzoyl chloride.

The *in vitro* antiprotozoal activity of compounds **1**-**13** was evaluated at the Laboratory of Microbiology, Parasitology and Hygiene (LMPH, University of Antwerp, Belgium) against red cell stages of *P. falciparum* K1, trypomastigotes of *T. b. brucei*, intracellular amastigotes of *T. cruzi* and *L. infantum* (Table **1**). Compounds **3** [8], **5** and **13** [9] and **6** and **9** [10] have already been described in the literature but their antiparasitic activity has never been investigated before.

Firstly, the presence of a halogen atom on the aromatic ring was essential for the inhibition of growth as 3α-(benzoyloxy)-tropane **13** did not exhibit any activity. Secondly, compounds **4**, **5**, **7** and **8** carrying chlorine or bromine in *ortho* and *meta* positions are more potent against *P. falciparum* whereas compounds **9** with a bromine in *para* position, **11** and **12** with a iodine in

Table 1. ***In vitro*** **activity (IC_{50} values as mean±SEM) of compounds 1-13 against** ***T. b. brucei (T.b.b.), T. cruzi (T.c.), P. falciparum (P.f.), L. infantum (L.i.)*** **and human fibroblasts MRC-5.**

Compound	*Ortho* [a]	*Meta* [a]	*Para* [a]	IC_{50} [b] (µM): mean ± SEM				
				T. b. b.	***T. c.***	***P. f.***	***L. i.***	***MRC-5***
1	2'-F			117.2±7.7	>197.5	19.1±2.4	>197.5	>197.5
2		3'-F		146.3±9.7	82.6±6.2	41.9±3.8	>197.5	>197.5
3			4'-F	30.6±2.3	116.3±7.9	24.1±1.7	>197.5	>197.5
4	2'-Cl			115.2±8.9	81.1±7.6	3.6±1.1	>197.5	>197.5
5		3'-Cl		28.8±3.1	29.6±2.1	8.1±1.3	116.3±8.4	133.5±9.9
6			4'-Cl	26.4±4.1	34.6±3.1	14.3±1.9	>197.5	>197.5
7	2'-Br			>197.5	>197.5	9.5±1.7	>197.5	>197.5
8		3'-Br		24.9±3.2	29.0±2.1	6.7±1.1	74.2±4.8	121.5±9.8
9			4'-Br	5.8±1.2	40.0±3.6	22.0±3.1	>197.5	>197.5
10	2'-I			61.6±4.9	63.1±4.7	14.4±2.8	>197.5	86.8±8.5
11		3'-I		5.6±1.9	25.4±3.3	35.8±6.1	21.7±4.5	65.0±3.9
12			4'-I	5.1±2.2	72.1±8.7	34.6±6.5	87.5±5.4	>197.5
13	2'-H	3'-H	4'-H	>197.5	>197.5	>197.5	>197.5	>197.5
suramin				0.03±0.04				
benznidazole					3.4±0.5			
artemether						< 0.01		
miltefosine							4.5±1.1	
tamoxifen								15.5±4.3

[a] Halogen position on the aromatic ring

[b] Half maximal inhibitory concentration (IC50) represents the concentration of drug able to inhibit *in vitro* growth by 50%.

meta and *para* positions, respectively, are more active against *T. b. brucei*, as reflected by IC_{50}-values below 10 µM. Against *T. cruzi* and *L. infantum*, compound **11** was the most active with IC_{50} of 25 µM and 22 µM, respectively. The activity varied with the substituted halogen and its position. Against *T. b. brucei*,

compounds with the heavier halogen iodine (IC_{50} = 5.1 µM for **12**) and bromine (IC_{50} = 5.8 µM for **9**) appeared to be more potent than those with chlorine (IC_{50} = 26.4 µM for **6**) or fluorine (IC_{50} = 30.6 µM for **3**). The position of the halogen also seemed to be important: higher activity against *T. b. brucei* when the halogen was in the *para* position, compared to *meta* and *ortho* positions (Table **1**). However, the *ortho* position (IC_{50} = 3.6 µM for **4**) instead of *para* position (IC_{50} = 14.3 µM for **6**) seemed more favorable for antiplasmodial activity. No cytotoxicity was reported except for 3α-(3'-chlorobenzoyloxy)tropane **5** (IC_{50} = 133.5 µM), 3α-(3'-bromobenzoyloxy)tropane **8** (IC_{50} = 121.5 µM), 3α-(2'-iodobenzoyloxy)tropane **10** (IC_{50} = 86.8 µM) and 3α-(3'-iodobenzoyloxy)tropane **11** (IC_{50} = 65.0 µM) which showed negligible toxicity against the human MRC-5 cell line. The absence of toxicity toward human cells is an essential requirement for the proper interpretation of *in vitro* screening results of a new drug [11].

Also crucial for *in vitro* activity towards *P. falciparum* and *T. b. brucei* is the incorporation of halogen atoms that increases the lipophilicity of the molecule and therefore improves membrane permeability. Consequently, oral absorption and blood brain barrier permeability may be enhanced [12, 13]. This latter property is a pre-requisite for potential drugs to cure cerebral malaria or sleeping sickness. The preliminary SAR described in this paper should encourage further studies aimed at improving the antiprotozoal activities of this class of compounds by optimization of substituents., as well as at investigating their mechanism of action.

It should be noted that the β-isomer of **3**, 3β-(4'-fluorobenzoyloxy)tropane also known as *p*-fluorotropacocaine (*p*FBT) acts as local anesthetic and anecdotally causes hypertension, tachycardia, anxiety and temporary psychosis [14].

3. CONCLUSION

These results confirm the importance of a halogenated atom and its position on the aromatic ring of the tropane skeleton for *in vitro* activity towards *P. falciparum* and *T. b. brucei*. The antiplasmodial activity increases with the halogen atom in ortho position whereas for *T. b. brucei*, the para position is the most appropriate for activity. In addition compounds with heavy halogens, like iodine or bromine, are more potent against *T. b. brucei* which may suggest a combination of electronegativity and hydrophobicity for

antitrypanosomal activity, as previously mentioned in the literature for *T. cruzi* [15]. 3α-(2'-Chlorobenzoyloxy)tropane **4** showed the most promising activity against *Plasmodium falciparum.*

In order to bypass the well-known effects of tropane alkaloids on the central nervous system, a series of quaternary amine analogs should be synthetized and evaluated in future work.

4. EXPERIMENTAL

High resolution mass (HRMS) analyses were recorded with a Waters Micromass-LCT Premier time-of-flight mass spectrometer (Milford, MA, USA) equipped with an electrospray ionization source in positive mode. NMR spectra were recorded in $CDCl_3$ on a Bruker AVANCE DRX 500 MHz instrument at 500 (^{1}H) and 125 MHz (^{13}C), using tetramethylsilane (TMS) as the internal standard. Chemical shifts are given in δ (ppm) scale and *J* values are given in Hz. Melting points (uncorrected) were obtained using a Büchi B-540 apparatus (Flawil, Switzerland). All reagents used were of analytical grade and obtained from Sigma-Aldrich.

4.1 Synthesis of 3α-(2'-fluorobenzoyloxy)tropane (1)

Tropine (0.79 mmol, 1 eq.) was dissolved in 5 ml of anhydrous tetrahydrofuran in a round-bottomed flask with molecular sieve (0.4 nm), then DMAP (0.04 mmol, 5 mol %) and triethylamine (0.58 mmol, 2 eq) were added. The use of the catalyst allowed increasing the yield to 67-89% comparing with the synthesis previously described by Maksay *et al*. [9] in which a yield of 40-55% was obtained. An inert atmosphere was established and maintained by a continuous flow rate of nitrogen. The mixture was cooled in an ice-bath, and 2-fluorobenzoyl chloride (0.87 mmol, 1.1 eq) introduced dropwise. The reaction was left at room temperature, under agitation, for 2 hours, then diluted with a saturated solution of Na_2CO_3 (10 ml) and distilled water (20 ml), and subsequently extracted with chloroform (3 x 30 ml). The organic portion was dried with anhydrous sodium sulfate and concentrated under reduced pressure at 35°C. The residue was purified by flash chromatography using an RP18 cartridge (25-40 μm, 17 g) on a SPOT Flash system from Armen Instrument (Saint-Avé, France). Elution was performed using H_2O (+0.1% NH_3) / methanol (+0.1% NH_3) with a gradient of 95:5 to 0:100 in 25 min (flow rate: 10 ml/min) and detection was carried out by UV at 254 nm. Compound **1** was obtained with a yield of 69%. White solid. Mp. 71 °C. HRMS *m/z*

264.1344 ($C_{15}H_{18}FNO_2$: $[M + H]^+$, requires 264.1322); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.94 (m, 1H, H-6'), 7.52 (m, 1H, H-4'), 7.22 (m, 1H, H-5'), 7.14 (m, 1H, H-3'), 5.27 (t, 1H, ^3J = 5.4 Hz, H-3), 3.15 (br s, 2H, H-1 and H-5), 2.31 (s, 3H, H-8), 2.24 (dt, 2H, ^2J = -14.3 Hz, H_{eq}-2 and H_{eq}-4), 2.07 (m, 4H, H-6 and H-7), 1.86 (d, 2H, ^2J = -14.3 Hz, H_{ax}-2 and H_{ax}-4); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 163.81 (C=O), 160.91 (C-F), 134.37 (C-4'), 132.10 (C-6'), 123.99 (C-5'), 119.20 (C-1'), 117.10 (C-3'), 68.50 (C-3), 59.73 (C-1 and C-5), 40.39 (NCH_3), 36.61 (C-2 and C-4), 25.52 (C-6 and C-7).

4.2 Synthesis of 3α-(3'-fluorobenzoyloxy)tropane (2)

The synthesis of **2** was performed using the same method described for **1**. The product **2** was obtained with a yield of 73%. White solid. Mp. 78.5 °C. HRMS *m/z* 264.1346 ($C_{15}H_{18}FNO_2$: $[M + H]^+$, requires 264.1322); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.82 (d, 1H, ^3J = 7.8 Hz, H-6'), 7.69 (m, 1H, H-2'), 5.33 (t, 1H, ^3J = 5.4 Hz, H-3), 3.15 (br s, 2H, H-1 and H-5), 2.31 (s, 3H, H-8), 2.24 (d, 2H, ^2J = -14.3 Hz, H_{eq}-2 and H_{eq}-4), 2.07 (m, 4H, H-6 and H-7), 1.86 (d, 2H, ^2J = -14.3 Hz, H_{ax}-2 and H_{ax}-4); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 164.44 (C=O), 160.80 (C-F), 130.60 (C-1'), 130.10 (C-5'), 124.90 (C-6'), 121.20 (C-4'), 117.86 (C-2'), 68.70 (C-3), 59.74 (C-1 and C-5), 40.39 (NCH_3), 36.64 (C-2 and C-4), 25.79 (C-6 and C-7).

4.3 Synthesis of 3α-(4'-fluorobenzoyloxy)tropane (3)

The synthesis of **3** was performed using the same method described for **1**. Compound **3** was obtained with a yield of 79%. White solid. Mp. 79.3 °C. HRMS *m/z* 264.1340 ($C_{15}H_{18}FNO_2$: $[M + H]^+$, requires 264.1322); This compound has already been synthesized by Kavanagh et al. [8]. However, its NMR data are different from ours. For this reason we add our own data here.^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.05 (m, 2H, H-2' and H-6'), 7.14 (m, 2H, H-3' and H-5'), 5.26 (t, 1H, ^3J = 5.3 Hz, H-3), 3.18 (br s, 2H, H-1 and H-5), 2.33 (s, 3H, H-8), 2.25 (d, 2H, ^2J = -12.6 Hz, H_{eq}-2 and H_{eq}-4), 2.12 (m, 2H, H_{ax}-6 and H_{ax}-7), 2.05 (m, 2H, H_{eq}-6 and H_{eq}-7), 1.85 (d, 2H, ^2J = -12.6 Hz, H_{ax}-2 or H_{ax}-4); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 164.90 (C=O), 164.67 (C-F), 131.90 (C-2' and C-6'), 127.08 (C-1'), 115.60 (C-3' and C-5'), 68.30 (C-3), 59.77 (C-1 and C-5), 40.43 (NCH_3), 36.68 (C-2 and C-4), 25.82 (C-6 and C-7).

4.4 Synthesis of 3α-(2'-chlorobenzoyloxy)tropane (4)

The synthesis of **4** was performed using the same method described for **1**. The product **4** was obtained with a yield of 69%. White solid. Mp. 53.2 °C. HRMS *m/z* 280.1053 ($C_{15}H_{18}ClNO_2$: $[M + H]^+$, requires 280.1026); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.80 (d, 1H, ^3J = 7.7 Hz, H-6'), 7.45 (m, 2H, H-3' and H-5'), 7.34 (t, ^3J = 7.6 Hz, H-4'), 5.28 (t, 1H, ^3J = 5.5 Hz, H-3), 3.18 (br s, 2H, H-1 and H-5), 2.32 (s, 3H, H-8), 2.28 (d, 2H, ^2J = -14.6 Hz, H_{eq}-2 and H_{eq}-4), 2.05 (m, 4H, H-6 and H-7), 1.90 (d, 2H, ^2J = -14.6 Hz, H_{ax}-2 and H_{ax}-4); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 165.06 (C=O), 133.60 (C-Cl), 132.34 (C-4'), 131.13 (C-5'), 130.68 (C-1'), 68.90 (C-3), 59.74 (C-1 and C-5), 40.33 (NCH_3), 36.50 (C-2 and C-4), 25.66 (C-6 and C-7).

4.5 Synthesis of 3*α*-(3'-chlorobenzoyloxy)tropane (5)

The synthesis of **5** was performed using the same method described for **1**. The product **5** was obtained with a yield of 71%. White solid. Mp. 77.8 °C. For analytical data see [9].

4.6 Synthesis of 3*α*-(4'-chlorobenzoyloxy)tropane (6)

The synthesis of **6** was performed using the same method described for **1**. The product **6** was obtained with a yield of 77%. White solid. Mp. 82.9 °C. HRMS *m/z* 280.1044 ($C_{15}H_{18}ClNO_2$: $[M + H]^+$, requires 280.1026); For ^{1}H-NMR data, see Wallace et al. [10]. ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 165.01 (C=O), 139.29 (C-Cl), 130.78 (C-3' and C-5'), 129.26 (C-1'), 128.79 (C-2' and C-6'), 68.44 (C-3), 59.76 (C-1 and C-5), 40.40 (NCH_3), 36.62 (C-2 and C-4), 25.80 (C-6 and C-7).

4.7 Synthesis of 3*α*-(2'-bromobenzoyloxy)tropane (7)

The synthesis of **7** was performed using the same method described for **1**. The product **7** was obtained with a yield of 69%. Colorless oil. HRMS *m/z* 324.0609 ($C_{15}H_{18}BrNO_2$: $[M + H]^+$, requires 324.0521);^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.62 (d, 1H, ^3J = 7.6 Hz, H-6') 7.60 (d, 1H, ^3J = 7.6 Hz, H-3'), 7.33 (t, 1H, ^3J = 7.6 Hz, H-5'), 7.29 (t, 1H, ^3J = 7.6 Hz, H-4'), 5.30 (t, 1H, ^3J = 4.9 Hz, H-3), 3.74 (br s, 2H, H-1 and H-5), 2.86 (d, 2H, ^2J = -15.7, H_{eq}-2 and H_{eq}-4), 2.69 (s, 3H, H-8), 2.27 (d, 2H, ^3J = 8.4 Hz, H-6_{ax} and H-7_{ax}), 2.16 (m, 2H, H_{eq}-6 and H_{eq}-7), 2.08 (d, 2H, ^2J = -15.7 Hz, H_{ax}-2 and H_{ax}-4); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 165.24 (C=O), 134.36 (C-3'), 132.80 (C-4'), 132.08 (C-1'), 130.65 (C-6'), 127.39 (C-5'), 121.21 (C-Br), 66.60 (C-3), 60.79 (C-1 and C-5), 38.11 (NCH_3), 34.16 (C-2 and C-4), 24.68 (C-6 and C-7).

4.8 Synthesis of 3α-(3'-bromobenzoyloxy)tropane (8)

The synthesis of **8** was performed using the same method described for **1**. The product **8** was obtained with a yield of 67%. White solid. Mp. 86.1 °C. HRMS *m/z* 324.0496 ($C_{15}H_{18}BrNO_2$: $[M + H]^+$, requires 324.0521); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.16 (s, 1H, H-2'), 7.96 (d, 1H, ^3J = 7.9 Hz, H-6'), 7.70 (d, 1H, ^3J = 7.9 Hz, H-4'), 7.35 (t, 1H, ^3J = 7.9 Hz, H-5'), 5.26 (t, 1H, ^3J = 5.4 Hz, H-3), 3.18 (br s, 2H, H-1 and H-5), 2.33 (s, 3H, H-8), 2.25 (d, 2H, ^2J = -14.5 Hz, H_{eq}-2 and H_{eq}-4), 2.12 (m, 2H, H_{ax}-6 and H_{ax}-7), 2.04 (m, 2H, H_{eq}-6 and H_{eq}-7), 1.85 (d, 2H, ^2J = -14.5 Hz, H_{ax}-2 and H_{ax}-4); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 164.55 (C=O), 135.77 (C-4'), 132.78 (C-1'), 132.50 (C-2'), 130.01 (C-5'), 127.92 (C-6'), 122.55 (C-Br), 68.79 (C-3), 59.76 (C-1 and C-5), 40.49 (NCH_3), 36.69 (C-2 and C-4), 25.81 (C-6 and C-7).

4.9 Synthesis of 3α-(4'-bromobenzoyloxy)tropane (9)

The synthesis of **9** was performed using the same method described for **1**. The product **9** was obtained with a yield of 84%. White solid. Mp. 90.5 °C. HRMS *m/z* 324.0447 ($C_{15}H_{18}BrNO_2$: $[M + H]^+$, requires 324.0521); For ^{1}H-NMR data, see Wallace et al. [10]. ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 164.59 (C=O), 132.11 (C-3' and C-5'), 130.78 (C-2' and C-6'), 128.69 (C-1'), 126.50 (C-Br), 65.66 (C-3), 61.90 (C-1 and C-5), 38.25 (NCH_3), 35.20 (C-2 and C-4), 24.81 (C-6 and C-7).

4.10 Synthesis of 3α-(2'-iodobenzoyloxy)tropane (10)

The synthesis of **10** was performed using the same method described for **1**. The product **10** was obtained with a yield of 73%. Colorless oil. HRMS *m/z* 372.0500 ($C_{15}H_{18}INO_2$: $[M + H]^+$, requires 372.0382); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.98 (d, 1H, ^3J = 7.6 Hz, H-6'), 7.74 (d, 1H, ^3J = 7.6 Hz, H-3'), 7.40 (t, ^3J = 7.6 Hz, H-5'), 7.13 (t, ^3J = 7.6 Hz, H-4'), 5.24 (t, 1H, ^3J = 5.5 Hz, H-3), 3.11 (br s, 2H, H-1 and H-5), 2.28 (s, 3H, H-8), 2.05 (m, 6H, H_{eq}-2, H_{eq}-4, H-6 and H-7), 1.66 (d, 2H, ^2J = -14.5 Hz, H_{ax}-2 and H_{ax}-4); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 166.05 (C=O), 141.62 (C-6'), 132.68 (C-5'), 130.51 (C-4'), 135.60 (C-1'), 128.20 (C-3'), 94.38 (C-I), 69.43 (C-3), 59.97 (C-1 and C-5), 40.73 (NCH_3), 36.78 (C-2 and C-4), 25.99 (C-6 and C-7).

4.11 Synthesis of 3α-(3'-iodobenzoyloxy)tropane (11)

The synthesis of **11** was performed using the same method described for **1**. The product **11** was obtained with a yield of 72%. White solid. Mp 108.3 °C. HRMS *m/z* 372.0416 ($C_{15}H_{18}INO_2$: $[M + H]^+$, requires 372.0382); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.35 (s, 1H, H-2'), 7.96 (d, 1H, ^3J = 7.9 Hz, H-6'), 7.88 (d, ^3J = 7.9 Hz, H-4'), 7.19 (t, ^3J = 7.9 Hz, H-5'), 5.23 (t, 1H, ^3J = 5.5 Hz, H-3), 3.15 (br s, 2H, H-1 and H-5), 2.31 (s, 3H, H-8), 2.23 (d, 2H, ^2J = -15.1 Hz, H_{eq}-2 and H_{eq}-4), 2.02 (m, 4H, H-6 and H-7), 1.83 (d, 2H, ^2J = -15.1 Hz, H_{ax}-2 and H_{ax}-4); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 164.61 (C=O), 141.88 (C-4'), 138.69 (C-2'), 132.95 (C-1'), 130.35 (C-5'), 128.71 (C-6'), 94.15 (C-I), 68.99 (C-3), 59.99 (C-1 and C-5), 40.79 (NCH_3), 36.95 (C-2 and C-4), 26.04 (C-6 and C-7).

4.12 Synthesis of 3α-(4'-iodobenzoyloxy)tropane (12)

The synthesis of **12** was performed using the same method described for **1**. The product **12** was obtained with a yield of 81%. Light brown solid. Mp. 136.3 °C. HRMS *m/z* 372.0421 ($C_{15}H_{18}INO_2$: $[M + H]^+$, requires 372.0382); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.83 (d, 2H, ^3J = 8.6 Hz, H-2' and H-6'), 7.74 (d, 2H, ^3J = 8.6 Hz, H-3' or H-5'), 5.27 (t, 1H, ^3J = 5.3 Hz, H-3), 3.19 (br s, 2H, H-1 and H-5), 2.33 (s, 3H, H-8), 2.27 (d, 2H, ^2J = -14.8 Hz, H_{eq}-2 and H_{eq}-4), 2.12 (m, 2H, H_{ax}-6 and H_{ax}-7), 2.04 (m, 2H, H_{eq}-6 and H_{eq}-7), 1.84 (d, 2H, ^2J = -14.8 Hz, H_{ax}-2 and H_{ax}-4); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 165.35 (C=O), 137.81 (C-3' and C-5'), 130.85 (C-2' and C-6'), 130.29 (C-1'), 100.57 (C-I), 68.47 (C-3), 59.80 (C-1 and C-5), 40.44 (NCH_3), 36.63 (C-2 and C-4), 25.79 (C-6 and C-7).

4.13 Synthesis of 3α-(benzoyloxy)tropane (13)

The synthesis of **13** was performed using the same method described for **1**. The product **13** was obtained with a yield of 89%. White solid. Mp 41-42°C [10]. HRMS *m/z* 245.1416 ($C_{15}H_{19}NO_2$: $[M + H]^+$, requires 245.1416); For ^{1}H-NMR data, see Maksay et al. [9]. ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 165.59 (C=O), 132.11 (C-3' and C-5'), 130.78 (C-2' and C-6'), 130.69 (C-1'), 127.90 (C-4'), 65.66 (C-3), 60.10 (C-1 and C-5), 38.95 (NCH_3), 35.90 (C-2 and C-4), 26.81 (C-6 and C-7).

4.14 Stock Solutions and Test Plate Production

Stock solutions of the compounds were prepared in 100% DMSO at 20 mg/ml [11]. The final in-test concentration of DMSO was <0.7%. The assays were performed in 96-well plates (Greiner) at 4-fold dilutions in a dose-titration range of 64 µg/mL to 0.25 µg/mL. Dilutions were carried out by a programmable precision robotic station (BIOMEK 2000, Beckman, USA). Each plate also contained medium-controls (blanks: 0% growth), infected untreated controls (negative control: 100% growth) and reference controls (positive control) [16]. Tests were run in duplicate in two independent experiments. The integrated panel of microbial screens for the present study and the standard screening methodologies were adopted as described by Cos *et al.* [11].

4.15 *In vitro* Assay on Intracellular Amastigotes of *L. infantum*

Leishmania infantum MHOM/MA(BE)/67 is maintained in the golden hamster and spleen amastigotes are collected for infection. Primary peritoneal mouse macrophages (PMM) are used as host cells and collected 2 days after peritoneal stimulation with a 2% potato starch suspension. Assays are performed in 96-well microtiter plates, each well containing 10 µl of the compound dilutions to which 190 µl of macrophage-parasite inoculum (3.10^5 cells + 3.10^6 parasites/well // RPMI-1640 + 5% FCSi) was added. After 5 days incubation, total amastigote burdens (number of infected cells x mean number of amastigotes per cell) were microscopically assessed after Giemsa staining. The results are expressed as % reduction in parasite burden compared to untreated control wells and an IC_{50} (50% inhibitory concentration) is calculated. Miltefosine was included as the reference drug [11].

4.16 *In vitro* Assay on Intracellular Amastigotes of *T. cruzi*

Trypanosoma cruzi, Tulahuen LacZ, clone C4 (nifurtimox-sensitive) is maintained on MRC-5_{SV2} (human lung fibroblast) cells in MEM medium, supplemented with 200 mM L-glutamine, 16.5 mM $NaHCO_3$, and 5% FCSi. All cultures and assays were conducted at 37 °C under a 5% CO_2 atmosphere. Assays were performed in 96-well microplates, each well containing 10 µl of the compound dilutions to which 190 µl of MRC-5 cell/parasite inoculum (2.10^4 cells/ml + 2.10^5 parasites/ml) was added. Parasite growth was compared to untreated-infected controls (100% growth) and non-infected controls (0% growth) after 7 days incubation. Parasite burdens were assessed after adding the substrate CPRG (chlorophenol red-

ß-D-galactopyranoside): 50 µl/well of a stock solution containing 15.2 mg CPRG + 250 µl Nonidet in 100 ml PBS. The change in color was measured at 540 nm after 4 hours incubation at 37 °C. The results are expressed as % reduction in parasite burdens compared to control wells and an IC_{50} is calculated. Benznidazole was included as reference drug [11].

4.17 *In vitro* Assay on Trypomastigotes of *T. b. brucei*

Trypanosoma brucei brucei Squib 427 strain (suramin-sensitive) was maintained in HMI-9 medium, supplemented with 10% FCSi. Assays were performed in 96-well microtiter plates, each well containing 10 µl of the compound dilutions to which 190 µl of the parasite suspension (7.10^4 parasites/ml) was added. After 3 days incubation, parasite growth was assessed fluorimetrically after addition of resazurin. Fluorescence was measured (λ_{ex} 550 nm, λ_{em} 590 nm) after 24 hours at 37 °C. The results are expressed as % reduction in parasite growth/viability compared to control wells and an IC_{50} is calculated. Suramin was included as the reference drug [11].

4.18 In vitro* assay Red Cells Stage of *P. falciparum

Plasmodium falciparum K1 strain (resistant to chloroquine, pyrimethamine and cycloguanil) was maintained in RPMI-1640 medium supplemented with 0.37 mM hypoxanthine, 25 mM Hepes, 25 mM $NaHCO_3$, and 10% O^+ human serum together with 2% washed human O^+ erythrocytes. All cultures and assays were conducted under an atmosphere of 4% CO_2, 3% O_2 and 93% N_2. Assays were performed in 96-well microplates, each well containing 10 µl of the compound dilutions to which 190 µl of the malaria parasite inoculum (1% parasitaemia, 2% HCT) was added. After 72 h incubation, plates were frozen and stored at –20 °C. After thawing, 20 µl of each well was transferred into another plate together with 100 µl Malstat™ reagent and 20 µl of a 1/1 mixture of PES (phenazine ethosulfate, 0.1 mg/ml) and NBT (Nitro Blue Tetrazolium Grade III, 2 mg/ml). Change in colour was measured spectrophotometrically at 655 nm. The results are expressed as % reduction in parasitaemia compared to control wells. Artemether was included as reference drug [11].

***4.19 In vitro* Assay for Cytotoxicity on MRC-5_{SV2} Cells**

MRC-5_{SV2} cells were cultured in Earl's MEM + 5% FCSi for determination of cytotoxicity/selectivity. Assays were performed in 96-well microplates, each well containing about 10^4 cells/well. After 3 days incubation, cell viability was assessed fluorimetrically after addition of resazurin (λ_{ex} 550 nm, λ_{em} 590 nm). The results are expressed as % reduction in cell growth/viability compared to untreated control wells and a CC_{50} is determined. When the CC_{50} is lower than 10 µM, the compound is classified as highly toxic. Tamoxifen was used as reference [11].

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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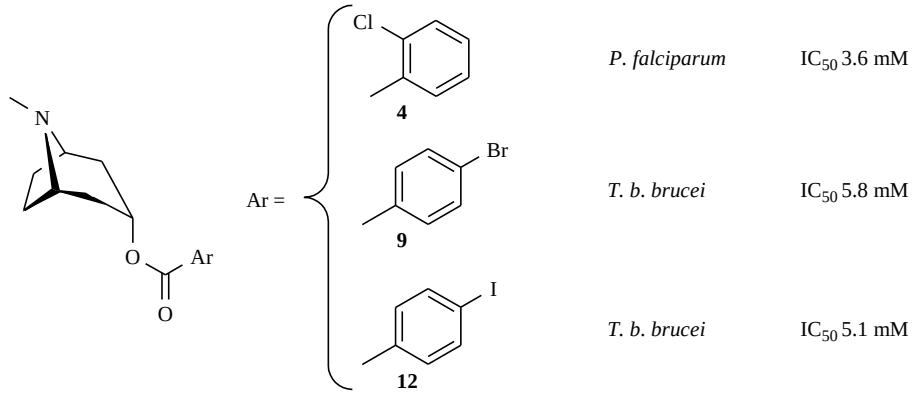
Graphical Abstract



Twelve halogenated tropane alkaloids were synthesized.

Their *in vitro* antiprotozoal activity was evaluated.