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Chemical constituents from *Waltheria indica* exert in vitro activity against *Trypanosoma brucei* and *T. cruzi*

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ABSTRACT

Six extracts from the roots and the aerial parts of *Waltheria indica* L. (Malvaceae) were screened for their in vitro antitrypanosomal activity towards *Trypanosoma brucei brucei* STIB 427 strain, *T. brucei rhodesiense* STIB 900 and *Trypanosoma cruzi* Tulahuen C4. The dichloromethane extract from the roots showed the highest activity against *T. cruzi* ($IC_{50} = 0.74$ μg/mL) as well as a good selectivity index (SI value of 35). Based on these results, this extract was fractionated and led to the isolation of three alkaloids (adouetin X (**1**), waltheriones A (**2**) and C (**3**)) and three pentacyclic triterpene derivatives (betulinic acid (**4**), 3β-acetoxy-27-*trans*-caffeoyloxyolean-12-en-28-oic acid methyl ester (**5**) and 3β-acetoxy-27-*cis*-caffeoyloxyolean-12-en-28-oic acid methyl ester (**6**)) identified by 1D and 2D NMR, UV, IR and MS analyses. Among these, waltherione C exhibited the highest and selective antitrypanosomal activity towards *T. cruzi* ($IC_{50} = 1.93$ μM) with low cytotoxicity ($IC_{50} = 101.23$ μM), resulting in a selectivity index value of 52. Waltherione C conforms to hit activity criteria with respect to *T. cruzi* as required by the WHO/TDR.

1. Introduction

American trypanosomiasis (AT) also known as Chagas disease is one of the most serious protozoan diseases which occurs throughout Latin America. The World Health Organization (WHO) estimated that 7 to 8 million people are infected worldwide, mostly in Latin America where Chagas disease is endemic [1]. Its etiological agent is *Trypanosoma cruzi*, a flagellate protozoa, which is transmitted to humans and other mammals mostly by the bite of a blood-sucking triatomine bug belonging to the *Reduviidae* family. To date, only two drugs, benznidazole and nifurtimox, are available on the market to treat Chagas disease. However these compounds provide unsatisfactory results for the chronic form and suffer from considerable side effects [2]. Consequently, there is a crucial need for new drugs which would ideally be affordable, safe and effective to treat this neglected disease.

Human African trypanosomiasis (HAT), also known as sleeping sickness, occurs in 36 sub-Saharan African countries and the number of cases is currently estimated to 20,000 [3]. The disease is caused by two sub-species of the kinetoplastid protozoan parasite *Trypanosoma brucei* (*T. b*): *T. b. gambiense* (West and Central Africa) and *Trypanosoma b. rhodesiense* (East Africa). Nowadays, only four drugs are licensed for the treatment of HAT: pentamidine, suramine, melarsoprol and eflornithine. As for Chagas disease, more effective treatments are needed. Another subspecies of the *Trypanosoma* genus is *T. brucei brucei* (*T .b. brucei*). This protozoan is pathogenic to wild and domestic animals and responsible for Nagana, also known as animal sleeping sickness. Nagana is a major obstacle to the economic development of affected rural areas [4].

In Africa, plants have been used traditionally for centuries and are still widely employed to treat sleeping sickness and Nagana. However, little is known about the treatment of HAT with plant-derived drugs and no trypanocidal molecule from natural origin is currently used against *T. brucei* infections [5]. Therefore, it is of prime importance to investigate plants used in African traditional medicine to treat parasitic diseases. One of them, *Waltheria indica* L. (Malvaceae), a shrub widespread in subtropical and tropical regions of the world, is commonly used in Burkina Faso to treat malaria [6]. In Niger/Nigeria, traditional healers give the whole plant to cattle as a tonic suggesting a possible activity against Nagana [7]. Prior work by our group focussed on the isolation and characterization of 10 quinoline alkaloids from *W. indica*, among which 8 compounds had not been described in the literature previously [8]. In the present work, further secondary metabolites with antitrypanosomal activity were identified through bioassay-guided fractionation. To achieve these objectives, extracts of various polarities were prepared from the roots and the aerial parts, and screened against the trypomastigote form of *T. b. brucei* (NYSM cell line) and *T. b. rhodesiense* (STIB 900), and the amastigote form of *T. cruzi* (Tulahuen

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C4). Six compounds, isolated from the dichloromethane extract of the roots which was the most active, were identified as adouetin X (**1**), waltherione A (**2**), waltherione C (**3**), betulinic acid (**4**), 3β-acetoxy-27-*trans*-caffeoyloxyolean-12-en-28-oic acid methyl ester (**5**) and 3β-acetoxy-27-*cis*-caffeoyloxyolean-12-en-28-oic acid methyl ester (**6**) (Fig. 1).

2. Materials and methods

2.1. General experimental procedures

Optical rotation was measured on a Jasco (Easton, MD, USA) P-1030 polarimeter (EtOH, *c* in g/100 mL). UV–vis spectra were recorded on a Perkin-Elmer (Wellesley, MA, USA) Lambda-25 UV–vis spectrophotometer in MeOH. IR spectra were measured on a Perkin-Elmer Spectrum 100. ^{1}H and ^{13}C NMR spectra were recorded on a Varian (Palo Alto, CA, USA) Unity Inova 500 MHz NMR instrument. HRMS spectra were obtained on a Micromass LCT Premier time-of-flight mass spectrometer (Waters, Milford, MA, USA) using electrospray in positive and negative modes. Capillary voltage was set at +2.8 kV and −2.4 kV, respectively, cone voltage at 40 V, source temperature at 120 °C, desolvation temperature at 250 °C, cone gas flow 20 L/h, and desolvation gas flow 600 L/h. UHPLC was performed on an Acquity UPLC System (Waters) with an Acquity BEH C_{18} column (1.7 μm; 50 × 2.1 mm i.d.; Waters). Medium pressure liquid chromatography was performed using a Shimadzu LC10AD pump equipped with a Knauer UV detector and an MPLC glass column (460 × 70 mm i.d.) loaded with ZEOprep C_{18} as the stationary phase (15–25 μm, Zeochem AG, Uetikon am See, Switzerland). Semi-preparative HPLC was performed on an ARMEN Spot Prep System (Saint-Avé, France) with a Kinetex Axia Core–Shell C_{18} column (5 μm, 250 × 21.2 mm; Phenomenex, Torrance, CA, USA).

2.2. Plant material

The aerial parts and the roots of *W. indica* were collected between June 2012 and February 2013 in Zinder (Niger). Their identification was confirmed by Didier Roguet (Botanical Garden of Geneva). Voucher specimens were deposited at the Botanical Garden of Geneva (No. 19003).

2.3. Preparation of the crude extracts

Air-dried powdered aerial parts (61 g) and roots (325 g) were extracted at room temperature (3 × 24 h) with 0.6 L and 3.25 L of CH_2Cl_2, respectively. After filtration, the solutions were evaporated to dryness and 6.0 g (9.8%) and 0.3 g (0.09%) of extracts were recovered, respectively. The plant residues (30 g of aerial parts and 160 g of roots) were then re-extracted with methanol three times as described above to get 2.8 g (9.2%) and 16.2 g (10.1%), respectively. Using the same protocol, aqueous extracts were obtained by maceration of 3 g of aerial parts and 4 g of roots in 30 mL and 40 mL of deionized water, respectively, to get 0.2 g (5.5%) and 0.7 g (18.0%) of extracts.

To isolate compounds of interest from the active dichloromethane root extract, air-dried roots (3.0 kg) were powdered and extracted with 3 × 10 L of CH_2Cl_2 for 24 h at room temperature. After filtration, the CH_2Cl_2 solutions were combined, evaporated to dryness and 2.3 g (0.07%) of a gummy residue was recovered.

2.4. UHPLC–PDA/MS analysis of the dichloromethane extract

The UHPLC–PDA/ESI/MS profiles were acquired using an Acquity UPLC System (Waters) equipped with an Acquity PDA detector and connected to a Quattro Micro triple quadrupole mass spectrometer (Waters) equipped with an ESI source operating in positive-ion mode. Samples were injected (2 μL) into a C_{18} Kinetex column (2.6 μm, 100 × 3 mm i.d.; Phenomenex) and eluted (0.5 mL/min, 40 °C) with ACN and H_2O both containing 0.1% formic acid. A gradient of 10 to 70% ACN in 40 min, then 70 to 98% in 5 min and followed by a washing step with 98% ACN for 5 min was used. After the washing step, the column was equilibrated with 10% ACN during 12 min before the next injection. The UV absorbance was measured at 210 nm, and PDA absorption spectra were recorded between 190 and 500 nm (1.2 nm steps). The conditions used for ESI/MS detection were: cone voltage, 30 V; capillary temperature, 350 °C; source voltage, 2.6 kV; nitrogen

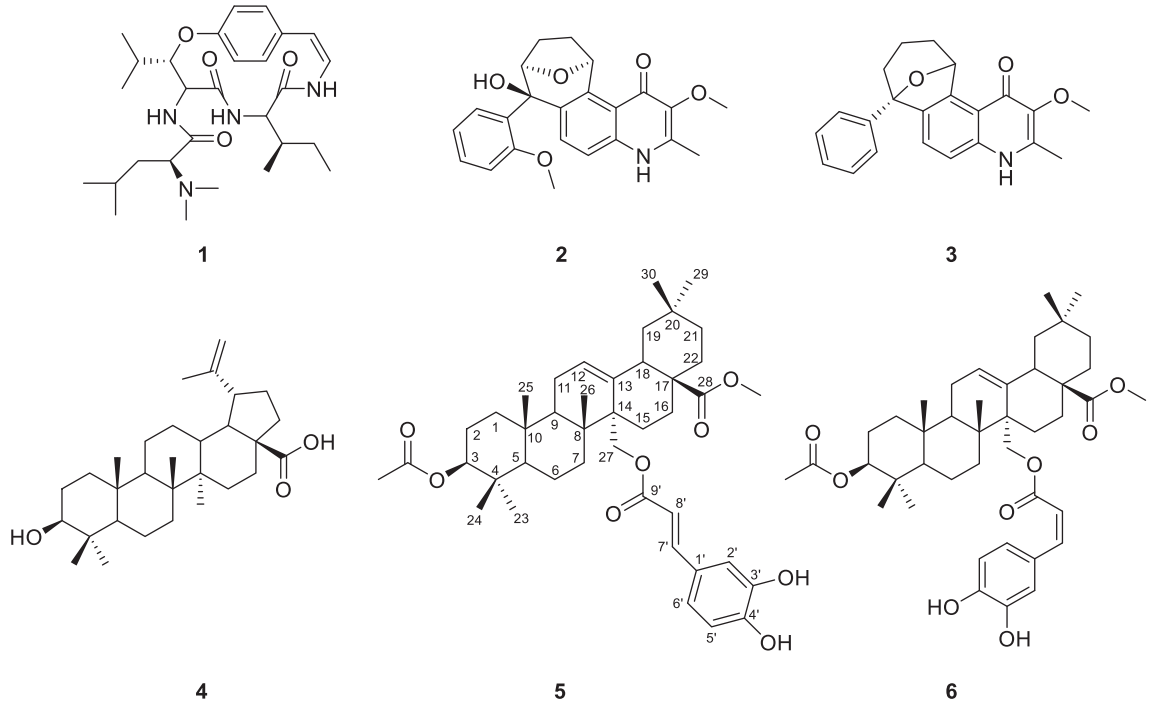

Fig. 1. Chemical structures of compounds **1**–**6**.