Fitoterapia 125 (2018) 55–58

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Antitrypanosomal activity and evaluation of the mechanism of action of diterpenes from aerial parts of *Baccharis retusa* (Asteraceae)

Anderson K. Ueno[a,1], Aline F. Barcellos[b,1], Thais A. Costa-Silva[b], Juliana T. Mesquita[c], Daiane D. Ferreira[c], Andre G. Tempone[c], Paulete Romoff[d], Guilherme M. Antar[e], João Henrique G. Lago[b,*]

[a] Institute of Environmental, Chemical and Pharmaceutical Sciences, Federal University of São Paulo, São Paulo 09972-270, Brazil
[b] Center of Natural Sciences and Humanities, Federal University of ABC, São Paulo 09210-580, Brazil
[c] Centre for Parasitology and Mycology, Instituto Adolfo Lutz, São Paulo 01246-000, Brazil
[d] School of Engineering, Mackenzie Presbyterian University, São Paulo 01302-907, Brazil
[e] Department of Botany, Institute of Biosciences, University of São Paulo, São Paulo 05508-090, Brazil

ARTICLE INFO

Keywords:
Baccharis retusa
Trypanosoma cruzi
Plasma membrane permeability
ent-kaurane diterpenes
ent-15β-senecioyl-oxy-kaur-16-en-19-oic acid

ABSTRACT

Baccharis retusa, a medicinal Brazilian plant from Asteraceae, has been used in Brazilian folk medicine to treatment of several illnesses, including parasitic diseases. Bioactivity-guided fractionation of the n-hexane extract from the aerial parts of *B. retusa* resulted in the isolation and characterization of three active related diterpenes: *ent*-15β-senecioyl-oxy-kaur-16-en-19-oic acid (**1**), *ent*-kaur-16-en-19-oic (**2**) and *ent*-16-oxo-17-nor-kauran-19-oic (**3**) acids. The structures of isolated compounds were defined by spectroscopic analysis, including NMR and HRESIMS. Antitrypanosomal activity of **1–3** was performed against cell-derived trypomastigotes using the colorimetric resazurin assay. The obtained results demonstrated that isolated compounds displayed a reduced toxicity against NCTC cells and were effective against the trypomastigote forms of *T. cruzi* with IC_{50} values of 3.8 μM (**1**), 75.3 μM (**2**) and 44.2 μM (**3**). Additionally, compound **3** displayed activity against amastigote forms of *T. cruzi* with IC_{50} of 83.2 μM. Compound **1** displayed the highest selectivity index (SI) when considered the trypomastigote forms, and its effect in the plasma membrane of parasite was evaluated using the fluorescent probe SYTOX Green. A considerable permeabilization (57%) in the membrane of the parasite was observed when compared to the untreated trypomastigotes. These data demonstrate, for the first time, the antitrypanosomal activity and mechanism of action of **1** and related compounds **2** and **3**, obtained from aerial parts of *B. retusa.*

1. Introduction

Trypanosoma cruzi is the etiologic agent of the Chagas disease, a hemoflagellate protozoan [1,2]. Recent estimates showed that there are 5.7–9.4 million people living with Chagas disease, leading cause of heart disease and cardiomyopathy, especially in Latin America and the United States. According to the non-profit organization Drugs for Neglected Diseases initiative (DNDi), in the next five years, 200.000 people living with Chagas disease will die from heart disease and related complications. DNDi also highlighted the urgent need to find better treatments [3]. Although Chagas disease has been described more than 100 years ago, the chemotherapy is, so far, limited to two nitro-heterocyclic drugs: benznidazole and nifurtimox [4]. Both are effective in the acute phase of the infection, with approximately 60–80% efficacy, but in the chronic phase exhibit reduced efficacy and severe side effects [4,5]. In this context, natural products have been providing interesting scaffolds for protozoan diseases.

Baccharis genus is represented by more than 500 species distributed primarily in the tropical areas of South America, including Brazil [6]. The genus accumulates several classes of natural compounds including trichothecenes [7], diterpenes [8,9], triterpenes [10,11] and flavonoids [7,12,13]. Biological studies have showed that crude extracts and isolated compounds from *B. dracunculifolia* displayed anti-*Trypanosoma cruzi* and anti-*Leishmania donovani* activities [11,14]. Previous studies demonstrated that sakuranetin (5,4′-dihydroxy-7-methoxyflavanone), a flavonoid isolated from aerial parts of *B. retusa*, showed anti-*T. cruzi* and anti-*Leishmania infantum* activities [13].

As part of our continuous studies concerning the selection of natural compounds from Brazilian flora biodiversity to discover new anti-protozoal agents [15,16], we present in this work the investigation of

* Corresponding author.
E-mail address: joao.lago@ufabc.edu.br (J.H.G. Lago).
[1] Both authors contributed equally to this work.

https://doi.org/10.1016/j.fitote.2017.12.016
Received 31 October 2017; Received in revised form 11 December 2017; Accepted 15 December 2017
Available online 18 December 2017
0367-326X/

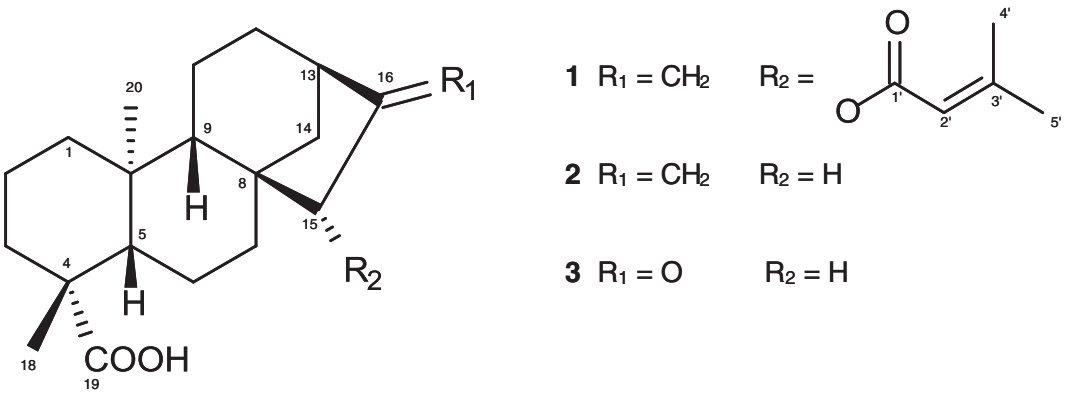

Fig. 1. Chemical structure of compounds **1–3** isolated from *B. retusa*.

Table 1
Anti-*T. cruzi* (trypomastigote and amastigote forms) and mammalian cytotoxicity (NCTC cells) of compounds **1–3**.

Compounds	IC_{50}/μM (95% CI)		CC_{50}/μM (95% CI)	SI	
	Amastigote	Trypomastigote	NCTC	Amastigote	Trypomastigote
1	NA	3.8 (3.2–4.5)	189.7 (164.8–219.9)	ND	50.0
2	NA	75.3 (55.3–87.3)	> 200	ND	2.6
3	83.2 (76.4–99.1)	44.2 (32.6–51.8)	> 200	2.4	4.5
Benznidazole	16.2 (12.9–20.3)	5.5 (3.5–8.6)	> 200	12.1	36.3

IC_{50}–50% inhibitory concentration, CC_{50} – 50% cytotoxic concentration, ND – not determined, NA – not active, SI – selectivity index, 95% CI – 95% confidence interval.

the anti-*Trypanosoma cruzi* effect against trypomastigotes and intracellular amastigotes of three related diterpenes: *ent*-15β-senecioyl-oxy-kaur-16-en-19-oic acid (**1**), *ent*-kaur-16-en-19-oic (**2**) and *ent*-16-oxo-17-nor-kauran-19-oic (**3**) acids. These compounds were isolated from n-hexane extract from aerial parts of *B. retusa* using a bioactivity-guided fractionation approach, being this the first occurrence of **3** as a natural product. Additionally, the interference in the plasma membrane of the compound with higher SI in trypomastigotes (**1**) was investigated using the vital dye SYTOX Green.

2. Results and discussion

Bioactivity-guided fractionation of the n-hexane extract from aerial parts of *B. retusa* led to the isolation of three active substances (**1–3**), as showed in Fig. 1. HRESIMS spectra of **1–3** showed *quasi*-molecular ion peaks $[M\text{-}H]^-$ at *m/z* 399.2541, 301.2191 and 303.1969, respectively, indicating the molecular formula $C_{25}H_{36}O_4$, $C_{20}H_{30}O_2$ and $C_{19}H_{28}O_3$ to each compound. Signals of double bonds at δ 156.9/155.7 (C-16) and 109.7/103.0 (C-17) as well as carboxyl group at δ 183.6/184.2 (C-19) were detected in the ^{13}C and DEPT 135 NMR spectra of the compounds **1** and **2**, respectively, indicating the occurrence of kaur-16-en-19-oic acid derivatives. In the case of **1**, additional peaks were observed at δ 166.7 (C-1′), 116.3 (C-2′), 155.7 (C-3′), 27.5 (C-4′) and 20.3 (C-5′), attributed to a senecioyl-oxy group which, due the presence of a signal at δ 81.9, was linked to C-15. Analysis of HMBC spectra associated to the value of optical specific rotation and comparison of spectral data described in the literature [17,18] allowed the identification of *ent*-15β-senecioyl-oxy-kaur-16-en-19-oic acid (**1**) and *ent*-kaur-16-en-19-oic acid (**2**). ^{13}C NMR and DEPT 135° spectra of **3** showed similarities of those recorded to compounds **1** and **2**, expect to the presence of 19 signals, indicating the occurrence of a nor-kaurane derivative. An additional peak at δ 222.5, assigned to the carbonyl group at C-16, was also observed in the ^{13}C NMR spectrum. Correlations observed in the HMBC spectrum and comparison of obtained data with those described in the literature [18] allowed the identification of **3** as *ent*-16-oxo-17-nor-kauran-19-oic acid.

Compounds **1–3** were incubated for 24 h with trypomastigotes forms of *T. cruzi* and the cell viability was determined by the resazurin assay. Compound **1** was the most effective diterpene against trypomastigote forms of *T. cruzi* with an IC_{50} value of 3.8 μM, while related derivatives **2** and **3** displayed IC_{50} of 75.3 and 44.2 μM, respectively. Only compound **3** displayed activity against the intracellular amastigotes, with an IC_{50} value of 83.2 μM. Benznidazole was used as the standard drug and showed IC_{50} values of 16.4 μM and 5.5 μM against trypomastigote and amastigote forms of *T. cruzi*, respectively. Only compound **1** presented moderate toxicity to NCTC mammalian cells, with a CC_{50} value of 189.7 μM while compounds **2** and **3** showed no toxicity up to > 200 μM. Benznidazole showed no cytotoxicity at the highest tested concentration (200 μM). The selectivity index (SI), given by ratio between the mammalian toxicity (CC_{50}) and the activity against the parasites (IC_{50}), resulted in a value of 50.0 for compound **1** (Table 1), indicating a superior selectivity than the positive control, benznidazole (36.3). Compounds **2** and **3** showed reduced activity and SI values of 2.6 and 4.5, respectively. Based on these data, our results indicated the important effect in the bioactivity caused by the presence of senecioyl-oxy group at C-15 in structure of compound **1**. Thus, the effect of compound **1** in the plasma membrane of *T. cruzi* was further investigated. The investigation of the lethal action of antitrypanosomal compounds may provide useful information regarding targets and potential metabolic pathways related to the anti-parasitic activity [19]. The parasite plasma membrane regulates the transport of nutrients, pH homeostasis, and homeostasis of other ions [20]. As could be seen in Fig. 2, compound **1** induced a considerable interference in the plasma membrane permeability (57%) of the parasite when compared to the untreated trypomastigotes. The results showed an intense and time-dependent increase in fluorescence levels when compound **1** was incubated with *T. cruzi* trypomastigotes for 120 min. Triton X-100 (0.5%) was used as a positive control, and it induced a maximal of plasma membrane permeabilization (100%). This effect could be possible ascribed to the lipophilic character of compound **1**, which may interact with the plasma membrane of the parasite. When tested against intracellular amastigotes of *T. cruzi*, compound **1** demonstrated lack of efficacy. Although the intracellular amastigotes are the most relevant stage of the parasite [3], the discovery of active compounds to combat