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Trypanocidal and toxicological assessment *in vitro* and *in silico* of three sesquiterpene lactones from Asteraceae plant species

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ABSTRACT

We report the effect of the Sesquiterpene Lactones Ambrosin, Incomptine B and Glaucolide E against seven strains of *Trypanosoma cruzi*, the etiological agent of Chagas Disease. These compounds were isolated from *Parthenium hysterophorus*, *Decachaeta incompta*, and *Vernonia liatroides*, respectively. We evaluated by flow cytometry the viability of epimastigotes. Ambrosin was the most effective, then Incomptine B, and Glaucolide E (IC_{50} = 67.1, 123.7, and 215.1 μM, respectively). These compounds were more potent than the drugs Benznidazole (IC_{50} > 400 μM) and Nifurtimox (IC_{50} = 199.7 to > 400 μM). Toxicity to mammalian Vero and Jurkat cells was also determined *in vitro*. All the compounds had a poor selective index (0.003–1.859). Toxicoinformatics is useful to forecast *in silico* toxicological and pharmacokinetic properties. Ambrosin and Incomptine B may not possess mutagenic, tumorigenic, or reproductive effects. Glaucolide E could possess a low mutagenic and high tumorigenic effects, and probably target the Amine Oxidase A, Prostaglandin and G/H Synthase I. Interestingly, Ambrosin, Incomptine B and Glaucolide E, comply with Lipinsky Rule of Five, indicating a suitable pharmacokinetic profile. Ambrosin and Incomptine B possess high trypanocidal activity, and pharmaceutical properties suitable for development; however, their safety profile should be optimized by structural modifications.

1. Introduction

Trypanosoma cruzi is the etiological agent of Chagas Disease, or American trypanosomiasis, which is transmitted to humans by the infected feces of insects from the Triatominae subfamily (World Health Organization, 2015). Although, it can also be transmitted by blood transfusions, organs transplantation, congenitally, and orally (Rueda et al., 2014; Silva-Dos-Santos et al., 2017). Chagas Disease is endemic for Latin America; however, human migration has spread the parasite to non-endemic countries, such as the United States, Canada, Spain and Australia (Basile et al., 2011; Jackson et al., 2014). Accordingly, to the World Health Organization, 5–6 million people are infected worldwide, and approximately 10,000 deaths per year are caused by *T. cruzi*; therefore, classifying Chagas Disease as the second prevalence

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infectious disease and cause of mortality associated with a vector-borne parasite (World Health Organization, 2015).

Nowadays, there are two approved drugs to treat Chagas Disease in clinics. However, it is recognized that Benznidazole (Radanilm™) and Nifurtimox (Lampit™) are mostly useful during the acute phase of the disease, and their administration during the chronic phase is highly controversial due to their relative inefficacy, toxicity, and side effects that force patients to abandon treatments (Cancado, 2002; Soares and Santos, 2009). Another factor hindering the treatment of Chagas Disease is the heterogeneous susceptibility and resistance of *T. cruzi* strains to Benznidazole and Nifurtimox (Grosso et al., 2010; León-Pérez et al., 2007; Martinez et al., 2013), which is highly associated with its great genetic variability (Campos et al., 2017). In fact, six *T. cruzi* Discrete Typing Units (DTU) named from TcI to TcVI are currently known (Zingales et al., 2012). DTU are highly important for the discovering, and development of novel trypanocidal compounds, that could be more effective with fewer side effects.

Natural products have proven to be a rich source of molecules for the discovery and development of new drugs (Newman and Cragg, 2016). In this context, Sesquiterpene Lactones (SL's) are promising compounds due to their great diversity of biological activities, such as, antiparasitic, antitumor, anti-inflammatory, analgesic, or cardiotonic properties (Chadwick et al., 2013; Chaturvedi, 2011). Many of them bear potential for the design of new antiprotozoal drugs (Karioti et al., 2007; Schmidt et al., 2002). However, many novel active compounds can show remarkable properties *in vitro*, but fail to reach clinics since their toxicological and pharmacokinetic properties are not adequate. These cases represent almost 40% of failures in clinical trials (Theodosiou et al., 2014). In order to reduce time and costs of drug discovery, toxicoinformatics provide remarkable tools to analyze and predict toxicological and pharmacokinetic properties of promising compounds (Theodosiou et al., 2014).

In light of this, it was interesting to examine the trypanocidal activity and toxicology of the SL's Ambrosin, Incomptine B and Glaucolide E isolated from several Asteraceae plant species. Incomptine A and B have proven to be active against *Entamoeba histolytica* and *Giardia lamblia*, as well as, against *Escherichia coli, Shigella sonnei*, and *S. flexneri* (Bautista et al., 2012; Calzada et al., 2009). Ambrosin in a 3:1 mixture with Artesovin is a potential antimicrobial agent due to their activity against *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Solujić et al., 2008). Glaucolide E has only been reported as able to relax uterine and aortic smooth muscle (Campos et al., 2003). We tested the trypanocidal activity of these compounds against seven *T. cruzi* epimastigotes strains of the DTUs TcI and TcVI, and compared with Benznidazole and Nifurtimox. As an early screening to assess their potential therapeutic use and safety profile, we determined the toxicity of these compounds to mammalian cells Vero and Jurkat *in vitro*, as well as, their toxicoinformatic and pharmacokinetic properties *in silico*.

2. Materials and methods

2.1. Plant material, compounds isolation and identification

The SL's Ambrosin, Incomptine B and Glaucolide E were isolated from the aerial parts of *Parthenium hysterophorus* L, *Decachaeta incompta* (DC.) R.M. King & H. Rob and *Vernonia liatroides* (DC.), as previously described (Calsteren et al., 2008; Calzada et al., 2009; Cespedes-Acuña et al., 2005). Voucher specimens are deposited at Herbariums IZTA (2694), MEXU (127886), and FCME (149867). The spectroscopic data of these compounds are in agreement with those previously reported. **Ambrosin.** m.p. 147–148 °C. **^{1}H NMR (300 MHz, CD_3OD)**. δ 1.05 (d, 3H, *J* = 7.4 Hz), 1.15 (s, 3H), 1.72 (m, 1H), 1.89 (m, 2H), 2.00 (m, 1H), 2.27 (m, 1H), 2.46 (m, 1H), 3.16 (m, 1H), 3.53 (m, 1H), 4.72 (d, 1H, *J* = 8.57), 5.63 (d, 1H, J = 3.21), 6.09 (dd, 1H, *J* = 5.85, 2.95 Hz), 6.22 (dd, 1H, *J* = 3.57, 0.5), 7.71 (dd, 1H, *J* = 5.88, 1.95). **^{13}C NMR (300 MHz, CD_3OD)**. δ 17.78 (C-11 and C-15), 25.47 (C-7), 30.75 (C-9), 35.00 (C-10), 45.85 (C-7), 49.15 (C-1), 57.5 (C-5), 82.06 (C-6), 120.65 (C-14), 131.28 (C-2), 140.16 (C-12), 167.05 (C-3), 172.91 (C-13), 213.8 (C-4) (Iskander et al., 1988). **Incomptine B.** m.p. 180–182 °C. **^{1}H NMR (300 MHz, $CDCl_3$)**. δ 1.32 (dd, 1H, J = 14.18, 1.96, H-9), 1.52 (s, 3H, H-14), 1.86 (d, 3H, J = 1.01, H-15), 2.53 (dd, 1H, J = 14.2, 4.2, H-9β), 2.82 (br s, 1H, H-7), 3.23 (dd, 1H, J = 7.5, 0.9, H-1), 4.17 (br s, 1H, H-8), 5.06 (dd, 1H, J = 10.62, 1.25, H-6), 5.28 (dd, 1H, J = 10.64, 1.28, H-5), 5.54 (dd, 1H, J = 11.47, 7.55, H-2), 5.75 (d, 1H, J = 1.78, H-13b), 6.10 (d, 1H, J = 11.48, H-3), 6.39 (d, 1H, J = 1.76, H13b). **^{13}C NMR (300 MHz, $CDCl_3$)**. δ 20.16 (C-14), 23.76 (C-15), 46.32 (C-9), 52.32 (C-7), 60.54 (C-1), 62.30 (C-10), 75.89 (C-6), 76.46 (C-8), 123.66 (C-13), 126.36 (C-5), 128.56 (C-2), 132.03 (C-3), 135.56 (C-4), 139.32 (C-11), 170.17 (C-12) (Bautista et al., 2012). **Glaucolide E.** m.p. 144–145 °C. **^{1}H NMR (300 MHz, $CDCl_3$).** δ 1.27 (bt, 1H, J = 11.6, H-3α), 1.38 (s, 3H, H-15), 1.92 (s, 3H, CH_3-2′), 2.02 (s, 6H, H-14 and CH_3-2Ac), 2.06 (s, 3H,CH_3-13Ac), 2.47 (d, 1H, J = 8.8, H-5), 2.58 (dd, 1H, J = 12.68, 6.64, H-3β), 2.67 (d, 1H, J = 12.67,H-9β), 3.00 (bt, 1H, J = 11.54, H-9α), 4.81 (s, 1H, H-6), 4.85 (d, 1H, J = 5.28, H-13), 4.99 (dd, 1H, J = 5.55, H-13), 5.02 (d, 1H, J = 2.92, H-8), 5.18 (d, 1H, J = 10.54, H-1), 5.58 (td, 1H, J = 10.5, 6.62, H-2), 5.68 (bs, 1H, H-3′b), 6.11 (bs, 1H, H-3′a). **^{13}C NMR (300 MHz, $CDCl_3$).** δ 17.75 (C-3′), 18.08 (C-14), 18.23 (C-2′), 20.87 (CH_3), 21.15 (CH_3), 42.62 (C-3), 45.90 (C-9), 56.30 (C-13), 59.67 (C-4), 66.50 (C-5), 68.43 (C-2), 69.69 (C-8), 82.32 (C-6), 127.28 (C-1), 127.53 (C-3′), 128.57 (C-11), 134.14 (C-10), 135.22 (C-2′), 164.23 (C-7), 166.11 (C-1′), 170.40 (C-12), 170.40 (CO), 170.53 (CO) (Calsteren et al., 2008).

2.1.1. Trypanosoma cruzi strains and treatments

The genotyped strains used in this study came from the culture collection of the American trypanosomiasis laboratory at the Instituto de Investigaciones Biomédicas, UNAM (Bosseno et al., 2002; Martinez et al., 2013). The Querétaro (TBAR/MX/0000/Querétaro) (TcI), Ninoa (MHOM/MX/1994/Ninoa) (TcI), JJO (MHOM/MX/0000/JJO) (TcI), Mor3 (MHOM/MX/1995/Mor003) (TcI) and Ver6 (MDID/MX/1991/Ver006) (TcVI) strains were isolated from humans, marsupials or vector insects from Mexico, whereas Silvio (TcI) and CL Brener (TcVI) are reference strains from humans and vector insects from Brazil. All the experiments were performed during the epimastigote stage of the parasite. And were grown in LIT medium (Liver Infusion Tryptose medium) supplemented with 10% FBS (Cat. 10500064, Gibco) and 25 μg/ml of hemin (H5533, Sigma-Aldrich), until the logarithmic phase of growth was reached. Subsequently, cells were resuspended in fresh LIT medium at a 1×10^6 parasites/ml density, then incubated at 28 °C and treated during 18 h with concentrations of 10, 50, 100, 200 and 400 μM of Ambrosin, Incomptine B, Glaucolide E, Benznidazole and Nifurtimox. For biological testing, the compounds were dissolved in DMSO as a vehicle, stored at 4 °C and protected from light. The final concentration of the DMSO was 1% in all the treatments.

2.1.2. Cell viability assay, flow cytometry and data analysis

The cell viability assays were performed with the LIVE/DEAD Viability/Cytotoxicity kit (Invitrogen) in accordance with the supplier's specifications, and previously experimental conditions to measure parasite viability were standardized (Supplemental Fig. S1). The parasites treated with the vehicle DMSO, Ambrosin, Incomptine B, Glaucolide E, Benznidazole and Nifurtimox were harvested and read in a FACSCalibur (Becton-Dickinson) flow cytometer. The FL1-H reading was performed at 530/30 nm for Calcein, and the FL3-H at 670 nm for Ethidium Homodimer-1. A total of 20,000 events were captured for each condition, and the data was analyzed with the FlowJo software V.10.0.7r2. All the assays were performed in triplicate.

2.1.3. In vitro cytotoxicity assay

Vero and Jurkat cells were cultivated in RPMI-1640 medium (ATCC 30–2001) or DMEM medium (ATCC 30–2002) and supplemented with 10% of F BS (ATCC 30–2020). For the assay, 1×10^5 cells/well were

seeded onto the test plates containing the pre-diluted compounds and incubated at 37 °C and 5% CO_2 for 48 h. Cells viability were determined fluorimetrically after addition of resazurin. Trypanocidal drugs Benznidazole and Nifurtimox were included as cytotoxic positive controls. For calculating selectivity index (SI), IC_{50} values > 400 μM were taken as being 400 μM. Selective activities of the compounds were calculated according to the formula: (SI) = IC_{50} Mammalian cells/IC_{50} Parasites.

2.1.4. In silico toxicology and pharmaceutical properties

OsirisData Warrior is an open source software, used to assess the possible toxicity risks, lipophilicity (expressed as clogP), solubility in water (expressed as logS), molecular weight, drug-likeness and drug scores taking in account the molecular structure of a compound, a detailed description of the algorithm can be found at Sander et al. (2015). PROTOX server was used to calculate molecular polar surface area (PSA), which is a good descriptor for characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability, blood–brain barrier penetration, and rodent oral toxicity (Drwal et al., 2014). Octanol/water partition coefficient (CLogP) was calculated by the methodology developed by Molinspiration as the sum of fragment-based contributions and correction factors, molecular polar surface area (PSA) and topological polar surface area (TPSA) were calculated as the sum of fragmented contributions based on the methodology published by Ertl and Schuffenhauer (2009). The Swiss Bioinformatic Institute web server was used to calculate absorption, distribution, metabolism and excretion (ADME) properties (Daina and Zoete, 2016). We used consensus Log P from 5 different predictions (iLOGP, XLOGP3, WLOGP, MLOGP, Silicos-IT); Log S (Silicos-IT) which is a fragmental method; Ghose improvement for Lipinski Rule of Five, and synthetic accessibility (Ghose et al., 1999).

2.1.5. Statistical analysis

All experimental values are expressed as the mean ± SEM of at least three independent experiments. For multiple comparisons of *Trypanosoma* cell viability, one-way ANOVA and Dunnett's tests were used. Ambrosin, Incomptine B, Glaucolide E, Nifurtimox and Benznidazole IC_{50} were calculated with the sigmoidal dose-response function, using at least three independent experiments. The IC_{50} from Ambrosin, Incomptine B and Glaucolide E were compared separately with Nifurtimox using extra-sum-square F test. Statistical significance was determined by $p \leq 0.01$. All calculations were performed using Graph Pad Prism V.6. (Graph Pad Software, San Diego, CA, USA).

3. Results

In the present study, we are reporting for the first time the trypanocidal, cytotoxic, toxicoinformatic and pharmacokinetic properties of the SL's Ambrosin, Incomptine B and Glaucolide E (Fig. 1). We evaluated their trypanocidal activity against epimastigotes of the Mor3, JJO, Ninoa, Silvio, Querétaro (TcI), Ver6 and CL Brener (TcVI) strains, by flow cytometry at different concentrations (Fig. 2 and Supplemental Fig. S1). The epimastigotes cell viability, and the IC_{50} were calculated for each strain (Table 1). The most active compound against all strains was Ambrosin with an average IC_{50} of 67.1 μM, and then followed by Incomptine B with 123.7 μM, and Glaucolide E with 215.13 μM. These compounds resulted more potent than the positive controls. Nifurtimox showed an IC_{50} ranging from 199.7 to > 400 μM. Benznidazole was not effective (> 400 μM), even though it is the first-line drug against *T. cruzi* (Fig. 2 and Table 1). Our results coincide with the reported natural resistance to Benznidazole against wild strains of *T. cruzi* (Mejia-Jaramillo et al., 2012; Wilkinson et al., 2008). Therefore, may explain the drug's therapeutic inefficacy in murine models (Molina et al., 2000; Urbina, 2009), as well as its questionable efficacy during the chronic phase of Chagas Disease (Morillo et al., 2015; Pérez-Molina et al., 2009).

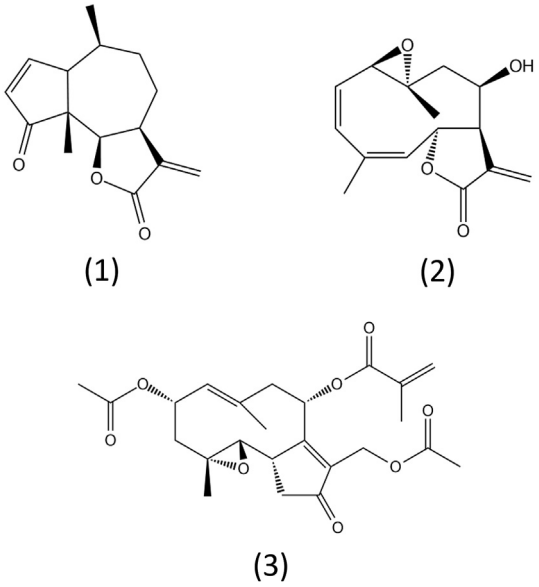

Fig. 1. Chemical structures of SL's Ambrosin (1), Incomptine B (2) and Glaucolide E (3), isolated from *Parthenium hysterophorus*, *Decachaeta incompta* and *Vernonia liatroides*, respectively.

Regarding the sensitivity of the *T. cruzi*'s DTU to the three SL's, the TcVI strains (Ver6 and CL Brener) were the most sensitive to Ambrosin and Incomptine B with an average IC_{50} of 21.52 ± 16.06 μM and 65.91 ± 3,67 μM, respectively. On the other hand, the TcI strains (Queretaro, Silvio, JJO, Mor3 and Ninoa) were the most resistant with an average IC_{50} of 84.91 ± 4.83 μM for Ambrosin, and 146.8 ± 8.96 μM for Incomptine B. However, the Glaucolide E showed an opposite performance, being most potent to TcI than to TcVI DTUs, although is necessary to complete the trypanocidal evaluation in all TcI strains (Table 1).

It is well known that many novel active compounds show remarkable properties *in vitro*, but fail to reach clinics since their toxicological and pharmacokinetic properties are not adequate. These cases represent almost 40% of failures in clinical trials (Theodosiou et al., 2014). In this context, we assessed the cytotoxicity of these compounds against Vero and Jurkat mammalian cell lines (Table 2 and Supplemental Fig. S2). Ambrosin, Incomptine B, and Glaucolide E had high toxicity to these cell lines (0.22–15.6 μM); however, Glaucolide E showed the lowest toxicity (IC_{50} > 400 μM) in Jurkat cells, even quite less than the drugs Benznidazole (IC_{50} = 295.6 μM) and Nifurtimox (IC_{50} = 179.5). Regarding to the Selectivity Index, all the compounds tested, including Benznidazole and Nifurtimox, had a poor performance (0.003–1.859).

As previously mentioned, the use of computer-aided drug discovery tools has shown to save time and money and to synergize the drug discovery process (Leelananda and Lindert, 2016), therefore in order to delve into the potential of the three sesquiterpene lactones as drug leads, we used toxicoinformatic tools to analyze and predict some of their pharmacokinetic and toxicological properties (Table 3). Interestingly, there is no prediction of any of these compounds to be substrate of P-glycoprotein (P-gp); this is an important result, since P-gp has been associated with drug resistance in protozoan parasites and reduces the efficacy of several drugs (Campos et al., 2003). On the other hand, the three SL's do not interact with any of the Cytochrome P450 (CYP) tested (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) (Table 3); interestingly CYP family is a major source of variability in drug pharmacokinetics and pharmacological response, and it has also been found to be involved as the main factor of the variability from treatment of infectious diseases (Bains, 2013).

From the toxicoinformatics perspective, two interesting forecasts arose. First, Ambrosin and Incomptine B may not possess mutagenic,

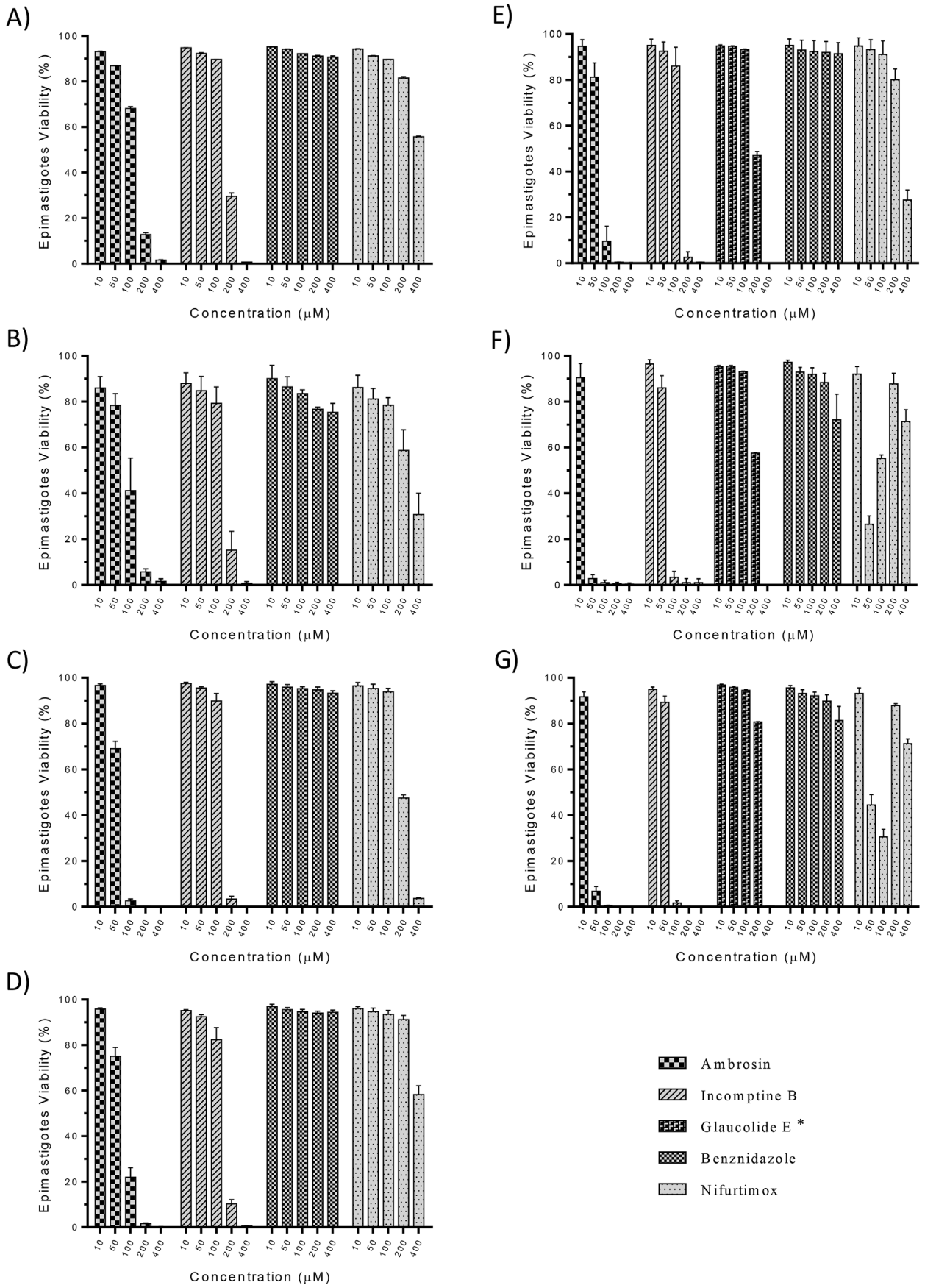

Fig. 2. Trypanocidal activity of SL's Ambrosin, Incomptine B and Glaucolide E, as well as positive controls Benznidazole and Nifurtimox in strains: Mor3 (A), JJO (B), Ninoa (C), Silvio (D), Querétaro (E), Ver6 (F) and CL Brener (G). * Evaluated for strains E to G.

Table 1
IC_{50} values calculated for SL's Ambrosin, Incomptine B and Glaucolide E, as well as positive controls Benznidazole and Nifurtimox in *T. cruzi* strains.

	IC_{50} [μM]				
Strain[DTUs]	Ambrosin	Incomptine B	Glaucolide E	Benznidazole [LCV]	Nifurtimox [LCV]
Silvio[TcI]	71.36[d]	139.8[d]	nd	> 400[94%]	> 400[58.3%]
Ninoa[TcI]	57.64[d]	135.5[d]	nd	> 400[93%]	199.2
Queretaro[TcI]	68.4[d]	132.3[d]	199.7[d]	> 400[91.5%]	318.2
JJO[TcI]	97.56[d]	152.2[d]	nd	> 400[75.4%]	300
Mor3[TcI]	129.6[d]	174.3[d]	nd	> 400[90%]	> 400[55.6%]
CL Brener[TcVI]	24.31[nd]	66.36[nd]	234[nd]	> 400[81.4%]	[b]
Ver6[TcVI]	18.73[nd]	65.46[nd]	211.7[nd]	> 400[72%]	[c]
Means	**67.10 ± 7.92**	**123.70 ± 6.85**	**215.13 ± 7.2**	—	—
Means for TcI	84.91 ± 4.83	146.8 ± 8.96	199.7 ± 1.4[a]	—	—
Means forTcVI	21.52 ± 16.06	65.91 ± 3.67	222.85 ± 10.1	—	—

Results represent the mean of three independent experiments.
[LCV]: Lowest Cell Viability.
[nd]: Not determined.
[a] IC_{50} of Querétaro strain as a representative of DTUs TcI strains.
[b] Biphasic effect of compound with 30.5% of cell viability at 100 μM (Maximum effect of compound).
[c] Biphasic effect of compound with 26.6% of cell viability at 50 μM (Maximum effect of compound).
[d] Significant difference ($p \leq 0.01$) versus control group Nifurtimox.

Table 2
Cytotoxicity (IC_{50} of cell viability) and selectivity index of SL's Ambrosin, Incomptine B, and Glaucolide E, as well as, of the drugs Benznidazole and Nifurtimox in Jurkat and Vero cell lines.

	IC_{50} [μM]				
Cell line	Ambrosin	Incomptine B	Glaucolide E	Benznidazole	Nifurtimox
Jurkat	0.22	15.6	> 400	295.6	179.5
SI	(0.003)	(0.123)	(1.859)	(0.739)	(0.512)
Vero	11.46	8.42	2.37	> 400	172.9
SI	(0.17)	(0.068)	(0.011)	(1)	(0.534)

SI: Selectivity Index = IC_{50} of mammalian cell viability/IC_{50} of epimastigote viability.

tumorigenic, or reproductive effects, and do not target any toxic receptor or possess any toxic fragment on their structures. In contrast, Glaucolide E possess a low mutagenic and high tumorigenic effects, and has the toxic targets Amine, Oxidase A, Prostaglandin and G/H Synthase I (Table 3). Second, Ambrosin and Incomptine B possess properties to be considered as highly irritant, and while Glaucolide E is considered as a low irritant. In a murine model toxicologic prediction algorithm, all the studied sesquiterpene lactones if swallowed, would fall in the category of harmful (LD_{50} < 2000 mg/kg), toxic (LD_{50} < 300 mg/kg), or fatal (LD_{50} < 50 mg/kg) (Table 3).

4. Discussion

The sesquiterpene lactones Ambrosin, Incomptine B and Glaucolide E (Fig. 1) were active against seven *T. cruzi* epimastigote strains, and resulted more potent than the current trypanocidal drugs used in clinics Benznidazol and Nifurtimox, but were also quite toxic to mammalian Vero and Jurkat cells. All the compounds tested showed a poor Selective Index (0.003–1.859). In the case of Ambrosin and Incomptine B due to their high cytotoxicity to mammalian cells, and in the case of the drugs, and Glaucolide E, due to their reduced activity to epimastigotes. Besides the sesquiterpene lactones here studied, others compounds of this class have shown antitrypanosomatid activity (Brengio et al., 2000; Jimenez-Ortiz et al., 2005; Saúde-Guimaraes et al., 2007; Schmidt et al., 2002; Sülsen et al., 2008). It is well known that the α-methylene-γ-lactone, and/or the α, β-unsaturated cyclopentenone rings of SL's, are responsible for most of their biological properties (Amorim et al., 2013), nevertheless, the structural geometry of the whole molecule is also important (Fabian et al., 2013; Schmidt et al., 2009). Cytotoxicity is mediated by an interaction of the α-methylene with sulfhydryl groups of enzymes involved in cellular metabolism (Hwang et al., 1996). In this context, Ambrosin and Incomptine B, that bear an α-methylene-γ-lactone, show high cytotoxicity to mammalian cells and epimastigotes. In the case of Glaucolide E that has a α, β-unsaturated cyclopentenone ring, showed reduced cytotoxicity to mammalian Jurkat cells and to epimastigotes.

The most promising targets for antiparasitic agents involve proteinases, enzymes of the glycolytic and pentose phosphate biosynthetic pathways, sterols and isoprenoids biosynthetic pathways and thiol-dependent redox metabolism; as well as poly-amine metabolism enzymes and purine salvage pathways (Duschak and Couto, 2007). It has been reported targets and mechanisms of action for specific SL's like Dehydroleucodine (DhL), Mexicanin I (Mxn), Helenalin (Hln), Psilostachyin (Psi) and Psilostachyin C (PsiC) (Barrera et al., 2013; Brengio et al., 2000; Jimenez-Ortiz et al., 2005; Jimenez et al., 2014; Schmidt et al., 2012). Brengio et al. (2000) demonstrated that DhL could be blocked by the presence of reducing substrates such as Glutathione (GSH) or Dithiothreitol (DTT), proposing that this compound interferes with the intracellular production of reduced GSH, which expose the parasites to damage by oxidative stress. Mxn and Hln also produce intracelular redox imbalance, but their mode of action seems different than that of the structuraly related DhL; it has postulated that either the cyclopentenone group present in Mxn and Hln or the spatial orientation of certain groups, e.g., hydroxyls or the α-methylene-γ-lactone itself, might be responsible for their trypanocidal effects (Jimenez-Ortiz et al., 2005; Schmidt et al., 2002) Artemisinin is another sesquiterpene lactone that is used as an antimalarial drug, which exerts its activity through heme binding (Schmidt et al., 2012). Another study performed with Psi and PsiC, isolated from plants of the genus *Ambrosia*, demonstrated these compounds possess different targets, on one side they are capable to inhibit the heme detoxification leading to the generation of oxidative stress in *T. cruzi* epimastigotes, and on the other side PsiC also interfered with the synthesis of sterols (Sülsen et al., 2016). Interestingly, both SL's induced parasite death by apoptosis. (Jimenez et al., 2014).

Toxicoinformatics can be a remarkable *in silico* tool useful to analyze and predict toxicological and pharmacokinetic properties for drug development. Despite their citoxicity to mammalian cells *in vitro*, pharmacokinetic and medicinal chemistry properties indicate that Ambrosin, Incomptine B and Glaucolide E, are in compliance with

Table 3
In silico toxicoinformatic and pharmaceutical analysis of Ambrosin, Incomptine B and Glaucolide E.

Prediction properties[a]	Ambrosin	Incomptine B	Glaucolide E
Physicochemical			
Lipophilicity (LogP)	2.06	1.66	1.29
Water Solubility (LogS)	−2.49	−1.51	−3.02
Topological Polar Surface Area (TPSA)	43.37 A	59.06 A	134.80 A
Rotatable Bonds	0	0	7
Number of H donors	0	1	0
Number of H-bond acceptors	3	4	10
Molar Refractivity	68.15	70.02	102.75
Molecular Weight	246.3 g/mol	262.3 g/mol	438.43 g/mol
Pharmacokinetic			
Gastro Intestinal Absorption	High	High	High
Blood Brain Barrier Permeability	Yes	Yes	Not
P-glycoprotein Substrate	Not	Not	Not
Cytochrome P450 1A2 inhibitor	Not	Not	Not
Cytochrome P450 2C19 inhibitor	Not	Not	Not
Cytochrome P450 2C9 inhibitor	Not	Not	Not
Cytochrome P450 2D6 inhibitor	Not	Not	Not
Cytochrome P450 3A4 inhibitor	Not	Not	Not
Skin permeation (Log Kp)	−7.07 cm/s	−7.08 cm/s	−9.29 cm/s
Medicinal Chemistry			
Lipinsky	Not violations	Not violations	Not violations
Ghose	Not violations	Not violations	Not violations
Bioavailability Score	0.55	0.55	0.55
Lead-likeness	Not, 1 Violation	Yes	Not, 1 Violation
Synthetic Accessibility[b]	4.34	5.14	5.79
Toxicoinformatic			
Toxicity Class[c]	Class III	Class IV	Class II
Mutagenic	None	None	Low
Tumorigenic	None	None	High
Irritant	High	High	Low
Reproductive effects	None	None	None
Possible Toxic Target	None	None	Amine Oxidase A Prostaglandin G/H Synthase 1
Toxic Fragments	None	None	None

[a] Predictions based on Osiris DataWarrior, Molinspiration, PROTOX and Swiss ADME web servers.
[b] From 1, very easy to 10, very difficult.
[c] Based on Toxic classes defined according to the globally harmonized system of classification of labeling of chemicals; where: Category I is fatal if swallowed (LD_{50} < 5 mg/kg), Category II is fatal if swallowed (LD_{50} < 50 mg/kg), Category III toxic if swallowed (LD_{50} < 300 mg/kg), Category IV harmful if swallowed (LD_{50} < 2000 mg/kg), Category V may be harmful if swallowed (LD_{50} < 5000 mg/kg), Category VI non-toxic (LD_{50} > 5000 mg/kg).

Lipinsky Rules of Five (Lipinski et al., 2001), indicating a suitable pharmacokinetic profile for therapeutics, and that they could be considered as leading compounds. These results are very positive since these rules are quite used to know the drug-likeness potential of novel compounds in the pharmaceutical industry, and represent an important step for the pharmaceutical development. Meaning adequate pharmacokinetic properties to reach the target, be bioactive in plasma and be easily formulated. Interestingly, these sesquiterpene lactones could be taken to inspire novel libraries of antiparasitic drugs or even to be tested in higher biological models of infection. In this context, all of them could be obtained in the laboratory since they possess a medium value of difficulty to be synthesized according to a synthetic accessibility value (Ertl and Schuffenhauer, 2009). Taking the above data into consideration, further studies are currently been performed in our laboratory in order to establish the molecular targets and mechanism of action, and toxicological risks that accounts for the trypanocidal activity of Ambrosin, Incomptine B, and Glaucolide E.

5. Conclusion

The sesquiterpene lactones Ambrosin and Incomptine B possess high trypanocidal activity, and pharmaceutical properties suitable for development; however, their safety profile should be optimized by structural modifications for the design of novel trypanocidal drugs.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2018.12.023.

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