ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

In Vitro, *In Vivo*, and *In Silico* Studies of Cumanin Diacetate as a Potential Drug against *Trypanosoma cruzi* Infection

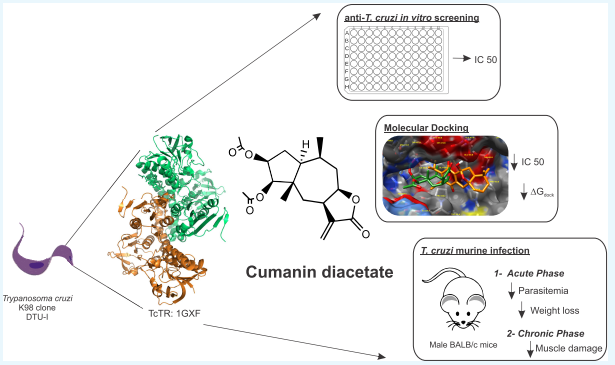
Andrés Sánchez Alberti,[○] María F. Beer,[○] Natacha Cerny, Augusto E. Bivona, Lucas Fabian, Celina Morales, Albertina Moglioni, Emilio L. Malchiodi, Osvaldo J. Donadel, and Valeria P. Sülsen*

Cite This: *ACS Omega* 2022, 7, 968–978

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: The sesquiterpene lactones cumanin, helenalin, and hymenin and their semisynthetic derivatives were evaluated against *Trypanosoma cruzi* epimastigotes. The cytotoxicity of the compounds was evaluated on murine splenocytes. Cumanin diacetate was one of the most active and selective compounds [IC_{50} = 3.20 ± 0.52 μg/mL, selectivity index (SI) = 26.0]. This sesquiterpene lactone was selected for its evaluation on trypomastigote and amastigote forms of the parasite. The diacetylated derivative of cumanin showed moderate activity on trypomastigotes (IC_{50} = 32.4 ± 5.8 μg/mL). However, this compound was able to efficiently inhibit parasite replication with an IC_{50} value of 2.2 ± 0.05 μg/mL against the amastigote forms. Cumanin diacetate showed selectivity against the intracellular forms of *Trypanosoma cruzi* with an SI value of 52.7. This cumanin analogue was also active on an *in vivo* model of Chagas disease, leading to a reduction in the parasitemia levels in comparison with nontreated animals. Histopathological analysis of skeletal muscular tissues from treated mice showed only focal interstitial lymphocyte inflammatory infiltrates with slight myocyte necrosis; in contrast, nontreated animals showed severe lymphocyte inflammatory infiltrates with necrosis of the myocytes. A molecular docking study of cumanin and its derivatives on trypanothione reductase from *T. cruzi* (TcTR) was performed. The results of ΔG docking achieved let the identification of diacetylated and *O*-alkylated derivatives of cumanin as good inhibitors of TcTR. Cumanin diacetate could be considered a potential candidate for further studies for the development of new therapies against Chagas disease.



1. INTRODUCTION

Sesquiterpene lactones (STLs) are natural terpenoid compounds that are mainly found in members of the Asteraceae family. They consist of 15-carbon structures with a γ-lactone ring with the presence, in many cases, of an exo-methylene group. A wide range of biological activities have been attributed to STLs. Among them, the antitumor, anti-inflammatory, and antiparasitic potential of this type of compounds stands out.[1]

Chagas disease or American trypanosomiasis is a vector-borne parasitic disease caused by the protozoan parasite *Trypanosoma cruzi*. This parasitosis affects 6−7 million people worldwide, and it is estimated that 75 million are at risk of infection. Chagas disease is endemic in Latin America, but nowadays has been spread to developing countries mainly due to migration phenomena.[2] The current therapy for Chagas disease is based on two drugs, benznidazole and nifurtimox. Even though these drugs are effective in the acute phase of the disease, the efficacy on chronic patients is still questionable. Furthermore, the lengthy treatment and side effects associated with these two drugs contribute to the lack of patient compliance and treatment interruption.

Trypanosoma cruzi is a kinetoplastid parasite of the Trypanosomatidae family. Several therapeutic targets have been identified in *T. cruzi*, which are considered useful for the development of new drugs: sterol biosynthesis,[3] purine salvage pathway,[4] trypanothione reductase,[5,6] cysteine proteinase,[7,8] trans-sialidase,[9] hypoxanthine-guanine phosphoribosyltransferase,[10] dihydrofolate reductase and glyceraldehyde-3-phosphate dehydrogenase,[11] uptake and degradation of heme pathway, among others.[12] Trypanothione reductase (TcTR) has been established as a validated target in *T. cruzi*. This enzyme is crucial for the parasite redox state, being essential for its survival. Apart from that, it is absent in the host, which makes

Received: October 6, 2021
Accepted: December 6, 2021
Published: December 20, 2021

it interesting for drug development.[13] The use of docking methods to identify potential candidates is a valuable tool extensively used for evaluating compounds and for guiding the synthesis of derivatives. Here, we report the *in vitro* and *in vivo* activities of the STL cumanin, helenalin, and hymenin and derivatives on *T. cruzi*. Considering that related compounds such as the terpenoid komaroviquinone have been studied as TcTR inhibitors,[14] a docking study was also performed to estimate the potential of the compounds as inhibitors of this enzyme too.

2. EXPERIMENTAL SECTION

2.1. Test Compounds. The STL cumanin (**1**) was isolated from *Ambrosia tenuifolia* Spreng. (Asteraceae) (BAF717). The diacetylated (**2**), silylated (**3**−**5**), and triazole derivatives of cumanin (**6**−**9**) were obtained and identified as previously described.[15]

Helenalin (**10**) and hymenin (**11**) were obtained from *Gaillardia megapotamica* var. *megapotamica* Spreng. (Asteraceae) (N°.4633) and *Parthenium hysterophorus* (Asteraceae) (1672-UNSL), respectively. Acetylated and silylated derivatives (**12**−**15**) were prepared from helenalin (**10**) and hymenin (**11**) as previously reported.[15]

2.2. Parasites. Epimastigotes of *T. cruzi* from RA strain were grown in a biphasic medium. Cultures were routinely maintained by weekly passages at 28 °C. *T. cruzi* bloodstream trypomastigotes (RA strain from discrete typing unit (DTU) VI and K98 clone, DTU I) as well as transfected trypomastigotes (Clone C4 of the Tulahuen strain, DTU VI) expressing β-galactosidase were obtained from infected CF1 mice.

2.3. Mice. Inbred BALB/c mice (male, 8 weeks old) were kept at the animal facility of Instituto de Microbiología y Parasitología Médica, IMPaM, Universidad de Buenos Aires-CONICET. Animal experiments were approved by the institutional animal care and use committee of the School of Medicine, UBA, Argentina (No. 2943/2013). All procedures were performed following the guidelines established by the National Research Council.[16] Animal sample size was estimated by a power-based method.[17]

2.4. In Vitro Anti-*T. cruzi* Assay. *T. cruzi* epimastigotes growth inhibition was evaluated by a [^{3}H] thymidine uptake assay.[18] Parasites were adjusted to a cell density of 1.5×10^6 parasites/mL and cultured in the presence of the natural compounds cumanin (**1**), helenalin (**10**), and hymenin (**14**) and derivatives (**2**−**9**, **11**−**13**, **15**) for 72 h at final concentrations ranging from 1.5 to 50 μg/mL. Compounds **10**−**13** were also evaluated in the range (1.50−0.01 μg/mL). Benznidazole (Active Pharmaceutical Ingredient) was used as a standard drug (Elea). The percentage of inhibition was calculated as 100 = {[(cpm of treated parasites)/(cpm of untreated parasites)] × 100}.

The trypanocidal effect of the STLs cumanin (**1**) and cumanin diacetate (**2**) was also tested on bloodstream trypomastigotes (RA).[18] Mouse blood containing trypomastigotes was diluted in RPMI medium to a cell density of 1.5×10^6 parasites/mL. Parasites were seeded by duplicate into a 96-well microplate with different concentrations of the STLs (0−100 μg/mL) and benznidazole. Plates were incubated for 24 h, and the remaining live parasites were counted on a hemocytometer. The percentage of live trypomastigotes was calculated as {[(live parasites after incubation)/(live parasites in untreated wells)] × 100}.

For the evaluation of cumanin (**1**) and cumanin diacetate (**2**) on intracellular forms of *T. cruzi*, 96-well plates were seeded with nonphagocytic Vero cells (ATCC CCL-81) at 5×10^3 per well in 100 μL of culture medium and incubated for 2 h at 37 °C in a 5% CO_2 atmosphere. The cells were washed and infected with Tulhauen bloodstream trypomastigotes expressing β-galactosidase at a parasite/cell ratio of 10:1. After 24 h of co-culture, the plate was washed twice with PBS to remove extracellular parasites and each compound and benznidazole was added at 0−100 μg/mL per well in 150 μL of fresh complete RPMI medium without phenol red. Nontreated cells (100% infection) and noninfected cells (0% infection) were used as controls. After 5 days, the cells were lysed with 1% Nonidet P-40 and chlorophenol red-β-D-galactopyranoside (CPRG) (100 μM) was added as β-galactosidase substrate. After 4−6 h incubation at 37 °C, the absorbance was measured at 570 nm in a microplate reader. The percentage of inhibition was calculated as 100 − {[(absorbance of treated infected cells)/(absorbance of untreated infected cells)] × 100}. The IC_{50}value was estimated as previously described.[18]

2.5. Cytotoxicity Assay. In a 96-well plate, spleen cells from BALB/c mouse (1.5×10^5) were incubated with different drug dilutions (200, 100, 50, 10, and 5 μg/mL) in RPMI medium containing 10% fetal calf serum. After 48 h of incubation at 37 °C (5% CO_2), the cells were harvested, washed once with PBS, and stained with 2.5 μg/mL propidium iodide (PI) for 5 min at room temperature. Subsequently, cell death was assessed by flow cytometry using a BD FACSaria II cytometer. Cells incubated only with drug vehicle were used as 100% viability control, and death percentage was calculated according to the following formula

$$\text{death}(\%) = \left[1 - \frac{(\%\text{PI}^- \text{cells})_{\text{drug-treated}}}{(\%\text{PI}^- \text{cells})_{100\%\ \text{viability control}}}\right] \times 100$$

Then, the concentration capable of causing cell death on 50% of splenocytes (CC_{50}) was determined using a nonlinear regression approach.

2.6. In Vivo Trypanocidal Activity. Mice were infected with 3×10^5 blood trypomastigotes of the K98 strain by intraperitoneal route. Parasitemia was measured weekly. Blood samples were diluted 1:5 in lysis buffer (0.75% NH_4Cl, 0.2% Tris, pH 7.2), and parasites were counted in a Neubauer chamber.

Mice were divided into groups of five to six animals each, and the drugs were administered by the intraperitoneal route (1 mg/kg of body weight/day) for five consecutive days after infection (11−15 dpi). Cumanin diacetate (compound **2**) and benznidazole were diluted with DMSO, and the concentration was adjusted with 0.1 M phosphate-buffered saline (pH 7.2). The vehicle was employed as a negative control.

2.7. Histopathological Analysis. Samples of heart and skeletal (quadriceps) muscles were dissected at 100 dpi and fixed with 4% formalin in PBS. The fixed tissue was embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The sections were analyzed at 200× and 400× magnification. A blind histological test was performed. Inflammation score semiquantitatively evaluated was determined according to the number and spreading of inflammatory foci graded as 1-focal, 2-multiple nonconfluent, 3- multiple confluent, and 4-multiple diffuse infiltrate.[19] The presence of necrotic areas was evaluated as well.

https://doi.org/10.1021/acsomega.1c05560
ACS Omega 2022, 7, 968−978

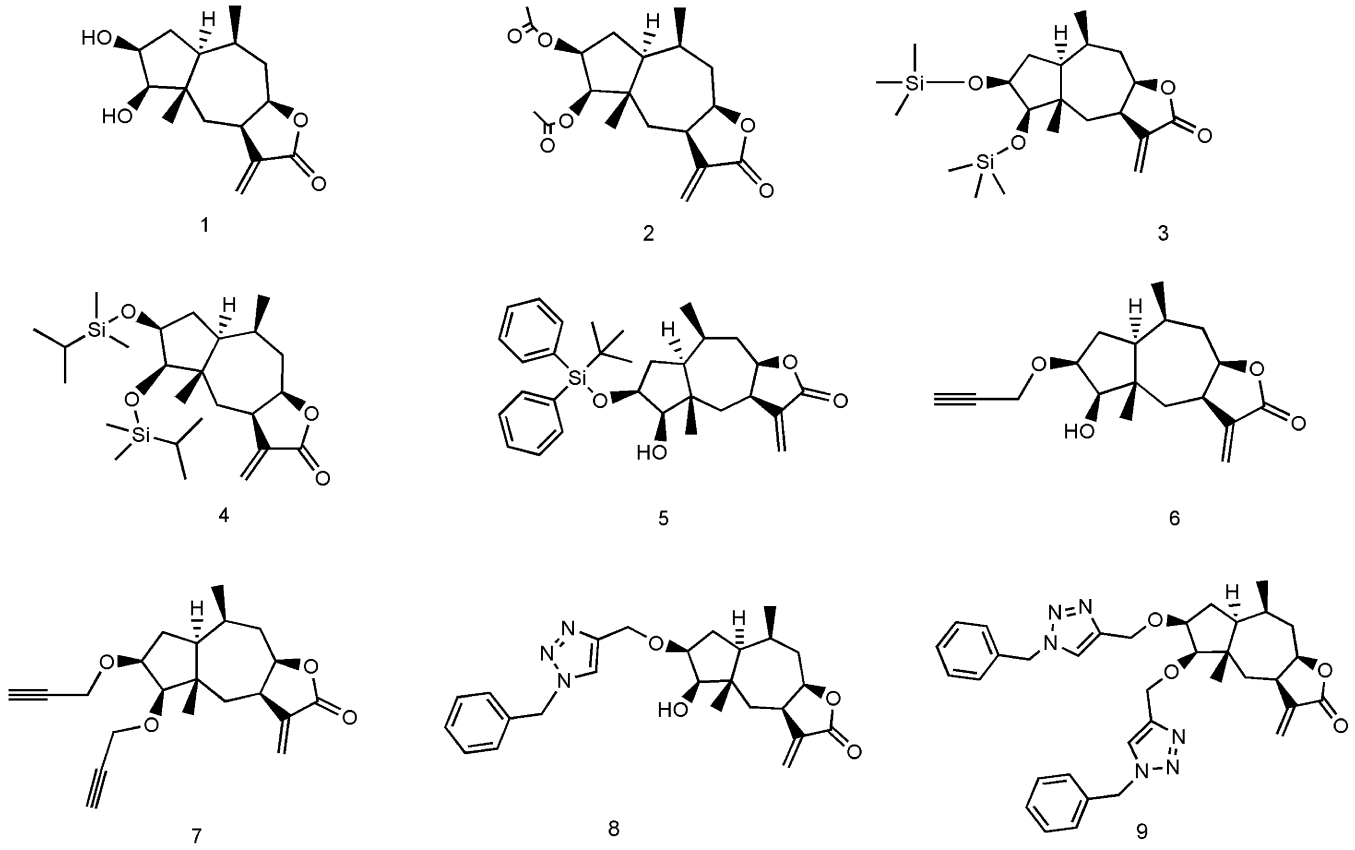


Figure 1. Chemical structures of cumanin (**1**) and derivatives (**2**–**9**).

2.8. Docking Study. The docking study with cumanin (**1**) and its derivatives (**2**–**9**) was performed using TcTR in its crystallographic form (PDB ID: 1GXF). It has a resolution of 2.7 Å and is complexed with two quinacrine molecules (inhibitor), FAD and maleic acid (MAE). The water molecules and the inhibitor molecules, FAD and MAE, were removed using the UCSF Chimera 1.9 program.

The ligand structures were built with the Hyperchem 8.0 program and optimized by means of the Polack-Ribiere algorithm, initially with the MM + method (gradient limit = 0.0001, stop cycles 9999) and then with the AM1 semi-empirical method (gradient = 0.001, stop cycles 9999). Finally, these structures were processed and prepared with the AutoDockTools 1.5.6 program (torsion adjustment, proton adjustment, and calculation of Gasteiger atomic charges).

The evaluation area (AutoDock grid box) was centered on the binding site of the inhibitor ligand (center of mass of both quinacrine molecules) and delimited in a volume of 66 × 66 × 66 $Å^3$ as indicated in the protocol used by Saha and Sharma,[14] comprising the amino acids: ALA342A, ARG355A, ARG472B, ASN23A, ASN340A, ASN456B, CYS469B, CYS53A, GLU19A, GLU466B, GLU467B, GLY459B, HIS461B, ILE-BLE107A, IU40339A, LULE33YSE PRO398B, PRO462B, SER15A, SER464B, SER470B, THR335A, THR355A, THR397B, THR457B, THR463B, TYR111A, VAL54A, VAL59A, ALA342A, ALA343A, ARG355A, ARG472B, GLB4U66B, AS45NU66B, GLB4YSA53, GLB4U66B, AS456-NYSA, GLB4YSA53, GLB4U667 GLY459B, HIS461B, ILE107A, ILE339A, ILE458B, LEU399B, LYS402B, LYS62A, PHE396B, PRO336A, PRO398B, PRO462B, SER15A, SER464B, SER470B, THR335A, THR455A, THR335A, THR455B, THR11R11R, THR455B, THR11R11R, TY455B, THR11R11R, TY455B, THR11R11R, TY455B, THR11R11R, TY455B.

The docking experiments were carried out using the programs AutoGrid 4.2, for the generation of the potential maps (based on the grid box), and AutoDock 4.2 for the conformational search and estimation of ligand−TcTR interaction energy (expressed as ΔG_{dock}, in kcal/mol). The genetic algorithm (GA) was used as the search method using the following configuration: number of final conformation (ga_run) 100; maximum number of evaluations (ga_num_evals) 25 000 000; maximum number of generations (ga_num_-generation) 27 000; mutation rate (ga_mutation_rate) 0.02; crossover rate (ga_crossover_rate) 0.8; local search on an individual in the population (ls_search_frequency) 0.06; number of top individuals to survive to next generation (ga_elitism) 1; maximum number of iterations per local search (sw_max_its) 300.

For the analysis of the results obtained with AutoDock 4.2, the tool package AutoDockTools 1.5.6 was used. To graph the interaction conformations (poses) chosen between ligand and TcTR, the UCSF Chimera 1.9 program was used.

2.9. Statistical Analysis. The results shown are presented as mean ± SD and mean ± SEM. GraphPad Software, Inc., (San Diego, CA) was employed to carry out calculations and IC_{50}. Statistical significance was determined by one-way analysis of variance (ANOVA) performed with software. Comparisons were referred to the control group unless otherwise indicated. p values < 0.05 were considered significant.

https://doi.org/10.1021/acsomega.1c05560
ACS Omega 2022, 7, 968−978

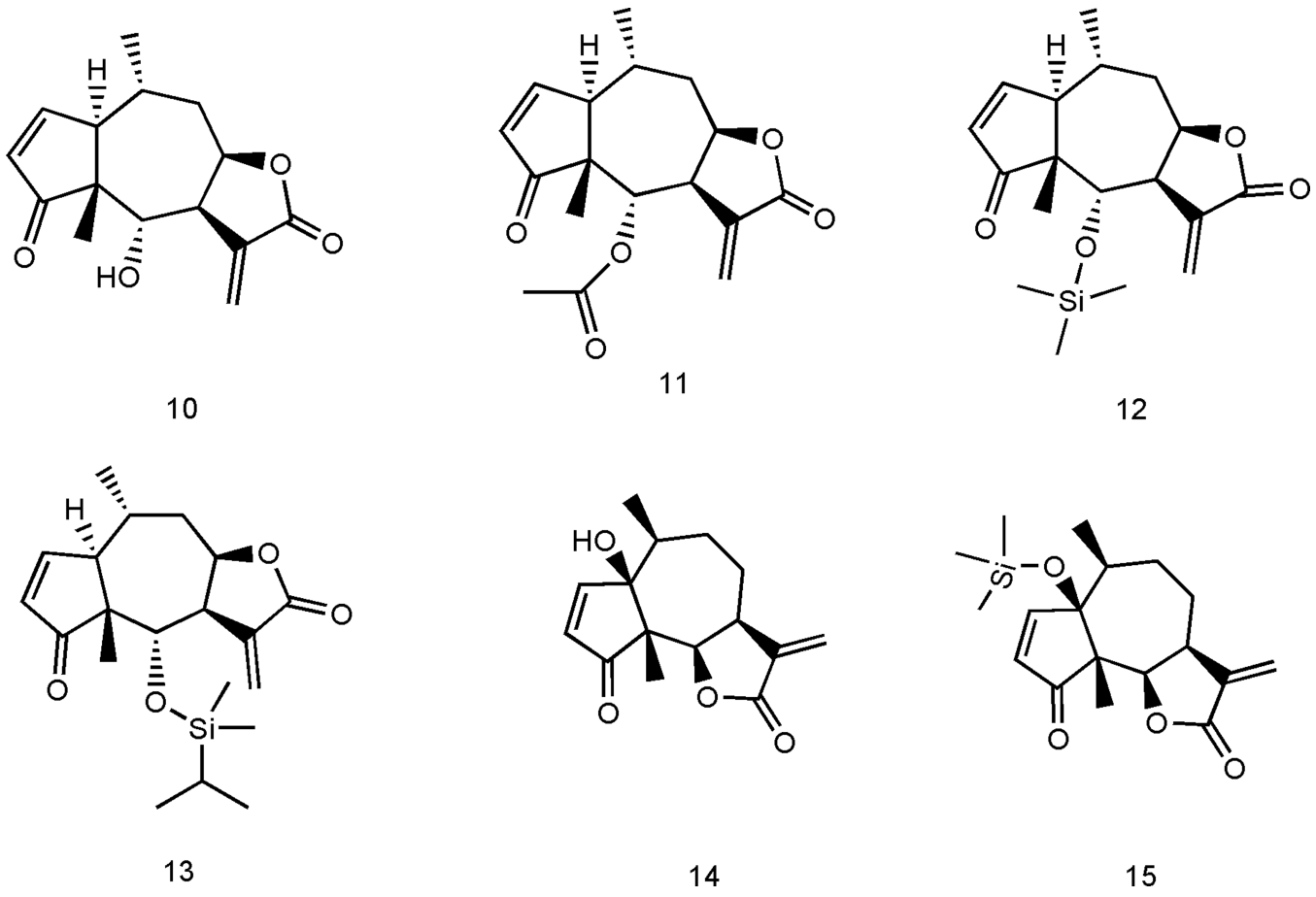


Figure 2. Chemical structures of helenalin (**10**), hymenin (**14**), and derivatives (**11**−**13**, **15**).

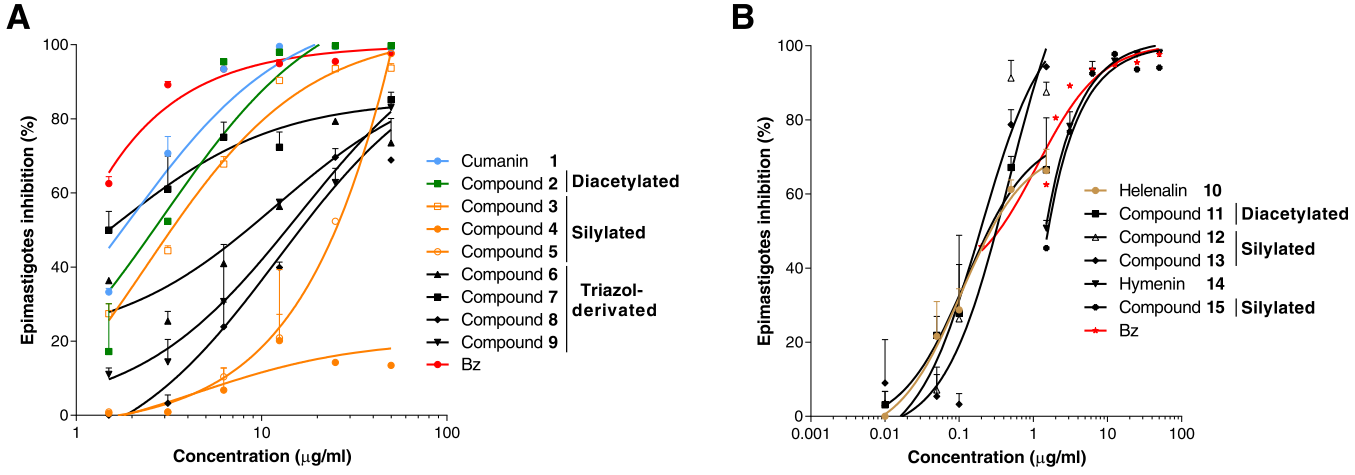


Figure 3. Effect of STLs and derivatives on *T. cruzi* epimastigotes. 1.5×10^6 parasites/mL were cultured in the presence of (**A**) cumanin (**1**) and derivatives (**2**−**9**) or (**B**) helenalin (**10**) and hymenin (**14**) and derivatives (compounds **11**−**13** and **15**, respectively) for 72 h. Assays were performed on a 96-well plate format, and 3H-thymidine was added during the last 16 h. Benznidazole (Bz) is shown as a reference drug. Symbols represent mean ± SD, and results represent at least three independent experiments.

3. RESULTS

The isolation of cumanin (**1**) and the syntheses of the diacetylated (**2**), silylated (**3**−**5**), and triazole derivatives (**6**−**9**) (Figure 1) have been previously reported.[15]

Helenalin (**10**) and hymenin (**14**) isolation, as well as the obtention of their derivatives (**11**−**13** and **15**) have been reported,[15] and their structures are shown in Figure 2.

3.1. In Vitro Activity on *T. cruzi* Epimastigotes.

The activity of cumanin (**1**) and its derivatives (**2**−**9**) on *T. cruzi* epimastigotes was evaluated by its ability to inhibit parasite proliferation employing [^{3}H]-thymidine as readout. The effect of compounds on the replication of the parasite is shown in Figure 3A.

The STL cumanin (**1**) was active on *T. cruzi* epimastigotes (IC_{50} = 2.98 ± 0.17 μg/mL), with an inhibition range of 33−

https://doi.org/10.1021/acsomega.1c05560
ACS Omega 2022, 7, 968−978

99% of parasite proliferation at the evaluated concentrations. From the series of cumanin derivatives, compounds **2** and **7** were the most active. They presented an IC_{50} value in the range of benznidazole, the benchmark drug (3.20 ± 0.52, 1.27 ± 0.41, and 1 ± 0.62 μg/mL, respectively).

Helenalin (**10**) proved to be highly active against epimastigotes (IC_{50} = 0.29 μg/mL), inhibiting 66% of parasite replication at a concentration of 1.50 μg/mL. Helenalin analogues, compounds **11**−**13**, presented IC_{50} values in the range of 0.18−0.29 μg/mL (Figure 3B). Both hymenin (**14**) and its silylated derivative (**15**) were active on epimastigotes with IC_{50} values of 1.50 and 1.70 μg/mL, respectively. The IC_{50} values of all compounds are shown in Table 1.

Table 1. IC_{50} Values on *T. cruzi* Epimastigotes, CC_{50} Values on Murine Primary Cultures, and Selectivity Indexes of Cumanin (1), Helenalin (10), Hymenin (14), and Derivatives[a]

compounds	IC_{50} (μg/mL) ± SD	CC_{50} (μg/mL) ± SD	SI
1	2.98 ± 0.17 (11.2)	7.8 ± 0.2 (29.4)	2.6
2	3.20 ± 0.52 (9.2)	84.4 ± 3.8 (240.8)	26
3	5.44 ± 0.19 (13.2)	27.3 ± 0.4 (66.4)	5.0
4	>50 (>107.1)	37.7 ± 0.5 (80.8)	n.d
5	23.66 ± 1.70 (46.9)	72.0 ± 1.5 (142.7)	3.0
6	7.61 ± 1.45 (25.0)	55.0 ± 5.4 (180.6)	7.2
7	1.27 ± 0.41 (3.7)	32.0 ± 8.9 (93.5)	25.2
8	14.97 ± 3.11 (34.2)	69.1 ± 12.0 (157.9)	4.6
9	13.11 ± 1.47 (21.5)	229.0 ± 4.0 (376.2)	17.5
10	0.29 ± 0.05 (1.11)	0.32 ± 0.3 (1.2)	1.1
11	0.27 ± 0.02 (0.74)	0.53 ± 0.2 (1.4)	2.0
12	0.29 ± 0.01 (0.95)	0.12 ± 0.1 (0.4)	0.4
13	0.18 ± 0.02 (0.53)	0.36 ± 0.3 (1.1)	2.2
14	1.50 ± 0.09 (5.8)	1.2 ± 0.8 (4.4)	0.8
15	1.70 ± 0.13 (5.1)	0.5 ± 0.7 (1.5)	0.3

[a]The values in parentheses correspond to the IC_{50} and CC_{50} expressed in μM.

3.2. Cytotoxicity. The cytotoxicity of the natural and semisynthetic compounds was determined on murine splenocytes. The CC_{50} values of the compounds are shown in Table 1. The cumanin derivatives presented CC_{50} values higher than the natural compound and in the range of 27−229 μg/mL. Helenalin (**10**) and its analogues showed CC_{50} values lower than 1 μg/mL on murine splenocytes. The CC_{50} values obtained for hymenin (**14**) and the silylated derivative (**15**) were 1.2 and 0.5 μg/mL, respectively.

The selectivity indexes (SI), defined as the relation between CC_{50} and IC_{50}, were calculated for each compound (Table 1). Cumanin derivatives **2**, **7**, and **9** were the most selective with SI values of 26, 25.2, and 17.5, respectively. Helenalin, hymenin, and their analogues showed low IC_{50} and CC_{50} values. The proximity of these values would be explained by the presence of a cyclopentenone ring, absent in cumanin and its analogues, which is highly susceptible to nucleophilic attack (SH, NH) and would determine a nonspecific mechanism. On the contrary, cumanin derivatives such as compounds **2**, **7**, and **9** showed certain selectivity (selectivity indexes in the range 17−26) that may indicate a more specific mode of trypanocidal action.

The STLs **1**−**9** were filtered through PAINS-Remover,[20] to detect possible pan assay interference compounds [PAINS]. This false-positive remover is recommended for exclusion in bioassays. All of the compounds (**1**−**9**) passed the filter.

3.3. In Vitro Activity on *T. cruzi* Trypomastigotes and Amastigotes. Taking into consideration that cumanin diacetate (**2**) was one of the most active and selective compounds against *T. cruzi*, epimastigotes, and its feasible chemical transformation from cumanin (**1**), both sesquiterpene lactones were assessed on bloodstream trypomastigotes and amastigotes (Figures 4 and 5).

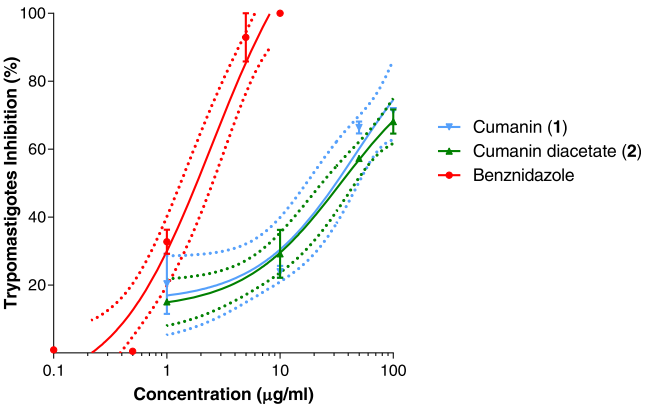


Figure 4. Effect of cumanin (**1**) and cumanin diacetate (**2**) on *T. cruzi* bloodstream trypomastigotes. Trypomastigotes of *T. cruzi* were cultured in the presence of either cumanin (**1**), cumanin diacetate (**2**) (1−100 μg/mL) or benznidazole (0.1−10 μg/mL) for 24 h and viable parasites were analyzed by optical microscopy. Lines represent nonlinear regression fitted by least squares; dotted lines represent 95% confidence bands. Results are expressed as mean ± SD and are representative of at least three independent experiments.

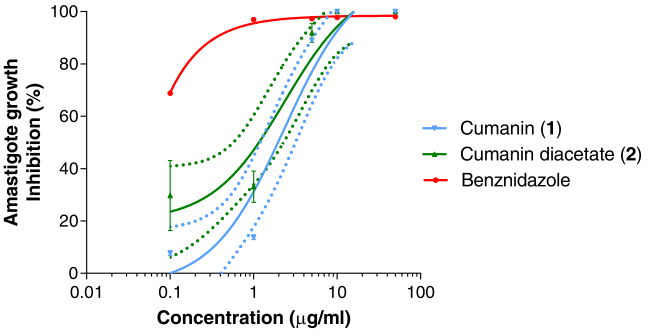


Figure 5. Effect of cumanin (**1**) and cumanin diacetate (**2**) on *T. cruzi* amastigotes. Infected cells were cultured in the presence of either cumanin (**1**), cumanin diacetate (**2**) (0.1−50 μg/mL), or benznidazole for 5 days. Results are expressed as mean ± SD.

Cumanin (**1**) and cumanin diacetate (**2**) showed moderate activity on *T. cruzi* trypomastigotes with IC_{50} values of 27.0 ± 2.6 (100.4 μM) and 32.4 ± 5.8 (92.5 μM), respectively. Against the amastigote forms, these compounds were able to inhibit the parasite replication with IC_{50} values of 2.2 ± 0.05 (8.3 μM) and 1.6 ± 0.2 (4.6 μM), respectively. Cumanin diacetate (**2**) showed selectivity against the intracellular forms of *T. cruzi* (SI = 52.7). The SI of the natural compound cumanin was 3.5, highlighting the improvement upon derivatization.

3.4. In Vivo Trypanocidal Activity of Cumanin Diacetate. Based on these results, cumanin diacetate (**2**) was selected for *in vivo* efficacy testing on a murine model of

https://doi.org/10.1021/acsomega.1c05560
ACS Omega 2022, 7, 968−978

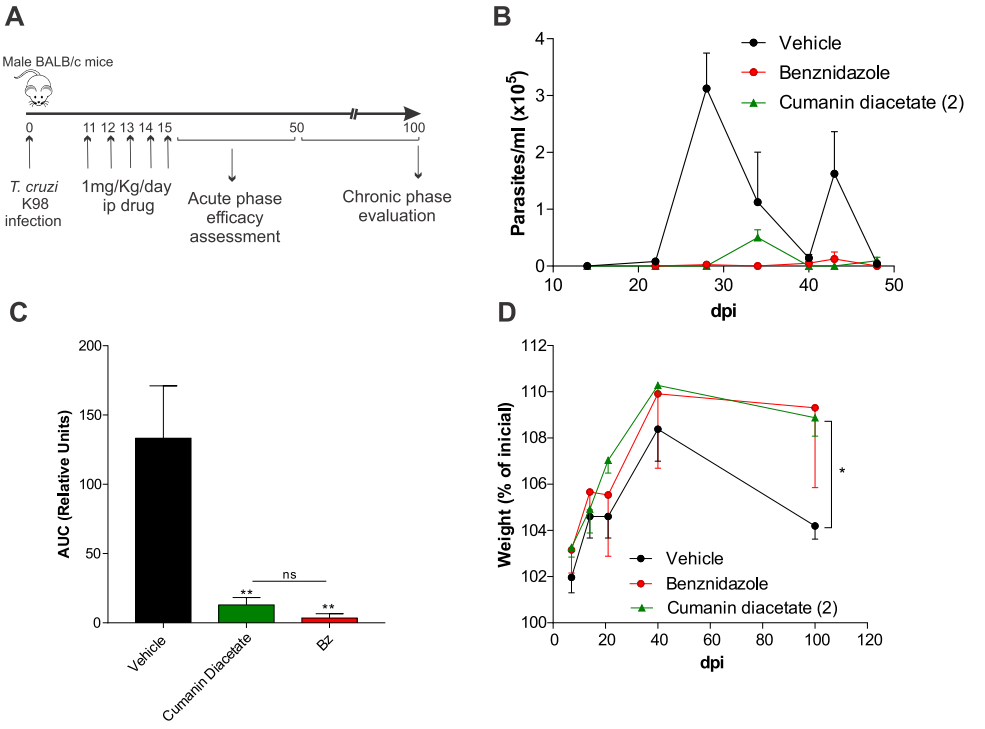


Figure 6. *In vivo* trypanocidal activity of cumanin diacetate. (**A**) BALB/c mice infected with 3×10^5 *T. cruzi* trypomastigotes were treated for five consecutive days (days 11 to 15 post-infection) with cumanin diacetate (**2**), beznidazole, or DMSO (vehicle, as control). (**B**) Parasitemia. (**C**) Area under the parasitemia curve (AUC) for each group. (**D**) Weight records after infection. Results expressed as mean±SEM are representative of two independent experiments. $^{**}p < 0.01$ against control mice, ns: non-significant.

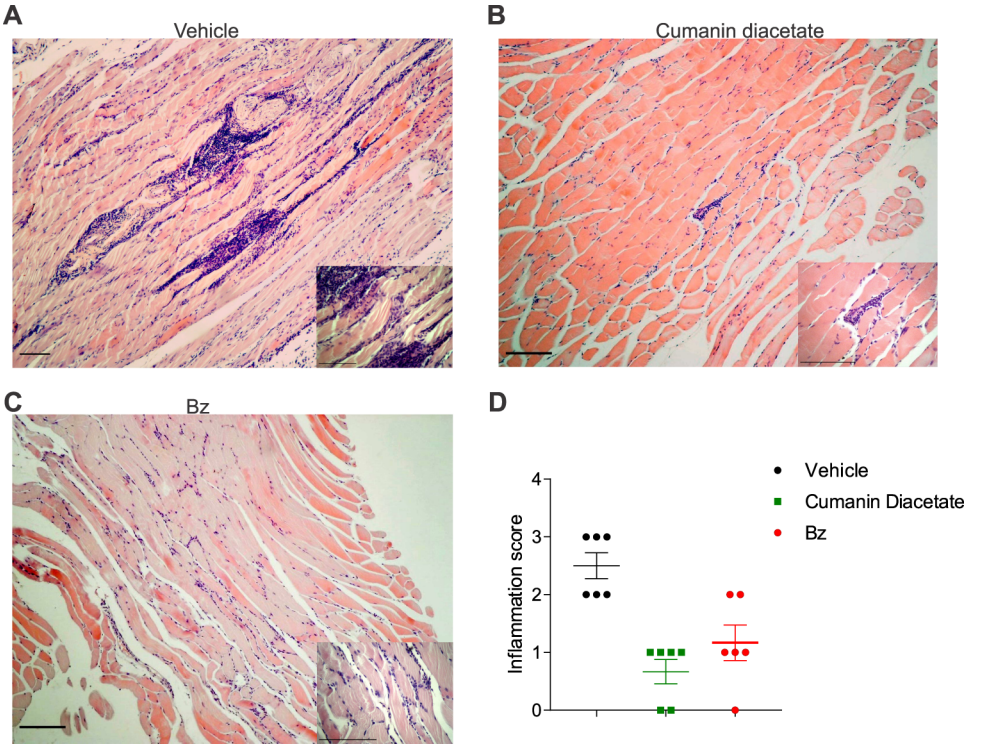


Figure 7. Tissue damage in vehicle and cumanin diacetate treated mice. Mice were infected with 300.000 trypomastigotes of the K98 strain and were treated intraperitoneally with cumanin diacetate between 11 and 15 dpi. Histopathological analysis of skeletal muscle was performed at 100 dpi. (**A**) Mock, (**B**) cumanin diacetate, and (**C**) benznidazole-treated mice. Insets show mononuclear cell infiltrates in each tissue. Bars indicate 100 μm. (**D**) Inflammation score. Samples were classified semiquantitatively for each individual according to the following scale of inflammatory infiltrates: (1) isolated foci; (2) multiple nonconfluent foci; (3) multiple confluent foci; and (4) multiple diffuse foci.

https://doi.org/10.1021/acsomega.1c05560
ACS Omega 2022, 7, 968−978

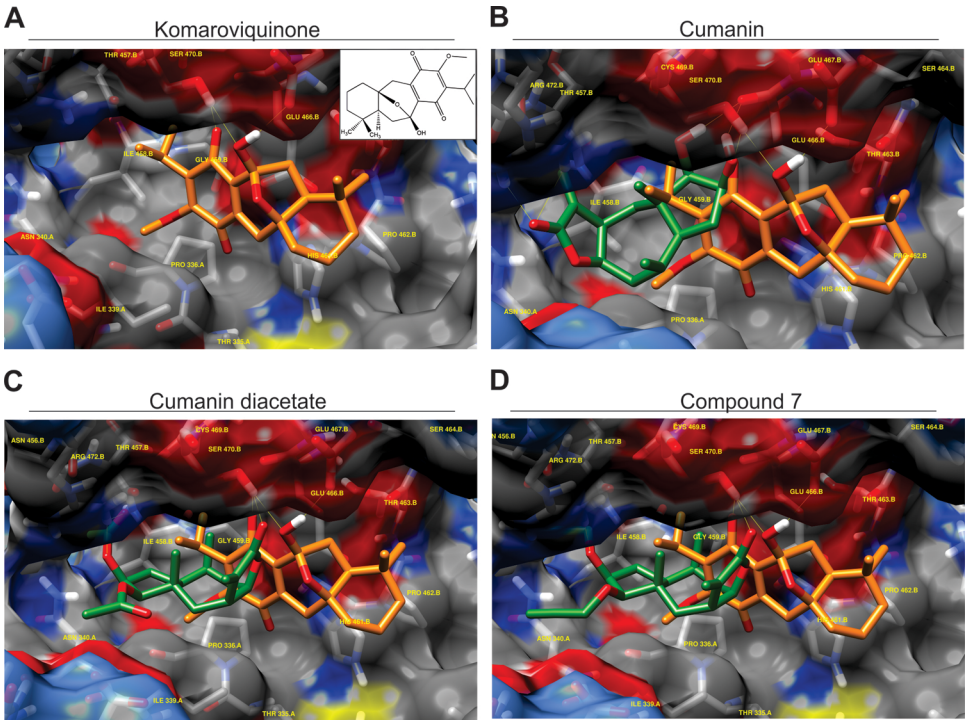


Figure 8. Docking results of selected compounds with TcTR. (**A**) Komaroviquinone; inset: chemical structure of this terpenoid. Binding poses of compounds (**B**) 1, (**C**) 2, and (**D**) 7 overlapped with Komaroviquinone. The yellow lines indicate hydrogen-bond interactions.

Chagas disease. BALB/c mice were infected with a myotropic strain of *T. cruzi* (K-98 clone), a parasite isolate useful for analyzing both acute and chronic phases of infection.[21] The mice were treated between days 11 and 15 post-infection with 1 mg/kg/day with either cumanin diacetate or benznidazole as a reference drug. The evolution of the parasitemia was assessed from day 13 to day 50 post-infection (Figure 6A).

T. cruzi infected mice that received only vehicle (control group) presented high levels of parasitemia and body weight loss at 100 dpi (Figure 6B−D). On the other hand, mice treated with cumanin diacetate showed a reduction of circulating parasites compared to the control group. In particular, treatment with this compound resulted in a 10-fold decrease in the area under the parasitemia curve (AUC) in comparison to mocked-treated mice. No significant difference was detected between treatment with benznidazole and cumanin diacetate (AUC: 4 *vs* 13, respectively, $p = 0.95$) (Figure 6C). Considering the variability in blood counts observed typically during the acute phase of *T. cruzi* infection, this indicator is an excellent metric of the overall parasitic load in blood during this moment.

Interestingly, at 100 dpi, animals treated with cumanin diacetate presented a significantly higher weight compared to the untreated animals (Figure 6D). To evaluate whether treatment with cumanin diacetate could reduce tissue damage characteristic of *T. cruzi* chronic infection, histological analyses of skeletal and cardiac muscle were performed on mice at endpoint (Figure 7).

No histopathological differences were observed in cardiac tissue in both control and treated mice, presenting in both cases foci of inflammatory infiltrate and necrosis of myocytes (data not shown). However, when analyzing skeletal muscle sections, an interstitial coalescent lymphocytic inflammatory infiltrate with necrosis of adjacent myocytes was observed in control animals (Figure 7A). On the contrary, animals treated with cumanin diacetate showed only focal interstitial lymphocytic inflammatory infiltrate with little necrosis of myocytes (Figure 7B) and similar inflammation score to mice treated with benznidazole (Figure 7C). These results highlight the efficacy of the treatment for improving pathology associated with the chronic infection.

3.5. Docking Studies on Cumanin Derivatives. All of the STLs included in this study have an α,β unsaturated carbonyl group in their structure. We have observed differences in activity and selectivity between cumanin and its derivatives (**1**−**9**) and helenalin and hymenin and analogues (**10**−**15**), suggesting for the last an unspecific mode of action. Although the trypanocidal activity of compounds **1**−**9** could be related to the interaction between the unsaturated γ-lactone and SH group of trypanothione,[22] the differences between IC_{50} and CC_{50} values for them would indicate a specific mode of trypanocidal action.

In an attempt to explain other possible mechanisms of action of cumanin and its derivatives and taking into consideration that STLs have shown interaction with TcTR,[12,22−25] a molecular docking study of compounds **1**−**9** was carried out. The criteria of including all cumanin series were based on the wide range of IC_{50} values obtained on *T. cruzi* epimastigotes (Table 1) for these compounds.

Komaroviquinone (K) was chosen as a model compound due to its terpenoid character similar to the STLs under study (Figure 8A, inset) and since it has shown trypanocidal activity against *T. cruzi* epimastigotes and has been proposed as an inhibitor of TcTR on an *in silico* study.[14]

The results of the experiment expressed as ΔG_{dock} (dock score, in kcal/mol) and Ki (expressed in μM) were reached considering as selection criteria the conformational cluster with the lowest energy (ΔG_{dock}) and the largest population, as summarized in Table 2. The interactions found for the poses of the selected ligands involved the following amino acids:

https://doi.org/10.1021/acsomega.1c05560
ACS Omega 2022, 7, 968−978

Table 2. Results of the Docking Experiment of Komaroviquinone (K), Cumanin (1), and Derivatives 2−9[a]

compound	log ($1/IC_{50}$)	ΔG dock (kcal/mol)	$K_{inhibition}$ (μM)	number of H bond	residues involved in H bond
K	5.43	−7.61	2.65	2	Glu467B, Ser470B
1	4.95	−6.92	8.49	3	Glu466B, Arg472B, Ser470B
2	5.04	−7.04	6.87	4	Arg472B, Ser470B
3	4.88	−6.94	8.21	2	Asn340A, Gly459B
4	3.97	−4.92	248.24	1	Gly459B
5	4.33	−6.28	24.82	1	Gly459B
6	4.60	−6.72	11.94	1	Arg472B
7	5.43	−7.58	2.78	2	Arg472B, Ser470B
8	4.47	−6.81	10.20	1	Leu399B
9	4.67	−6.95	8.08	2	Leu399B

[a]Komaroviquinone IC_{50} = 1.00 μg/mL ref 14.

ASN23A, ASN340A, ASP117A, ASP327A, CYS53A, GLU113A, GLU19A, GLU466B, GLY16A, GLY51A, LEU18A, ILE339, PRO336A, SER110A, SER116A, SER15A, THR335A, TYR111A, TYR22A, TYR455A, TYR52A, SER161A, THR457B, and GLU467B. Among them, ARG472B, ASN340A, GLU19A, GLU466B, GLU467B, GLY459B (backbone), ILE456B (backbone), and LEU399B (backbone) were found to intervene in the formation of hydrogen bond with the ligands studied (compounds **1**−**9** and komaroviquinone). The hydrogen bridge interactions (yellow lines) between the mentioned amino acids and cumanin (**1**), cumanin diactetate (**2**), and derivative **7** are shown in Figure 8.

The correlation between the energies predicted by the docking score (ΔG_{dock} in kcal/mol) and the anti-*T. cruzi* activity on epimastigotes of compounds **1**−**9** was analyzed (Figure 9). The correlation between Log ($1/IC_{50}$) and ΔG_{dock} (n = 10) showed a squared Pearson correlation coefficient (R^2) = 0.79, standard deviation SD = 0.21 and Fisher significance test (F) = 36.36 at significance (αF) = 0.00031. These results encourage the hypothesis of the possible mechanism of action of these STLs as TcTR inhibitors.

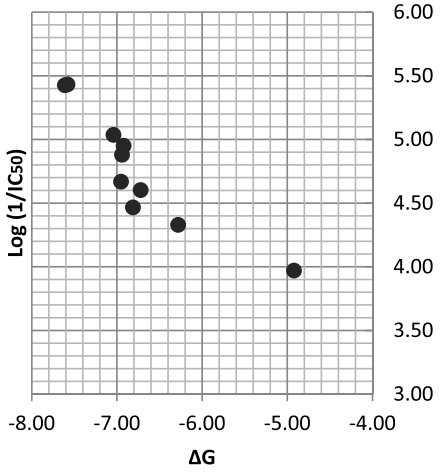


Figure 9. Correlation between trypanocidal activity [Log ($1/IC_{50}$)] (in molar) and docking performance [ΔG_{dock} (in kcal/mol)] for compounds **1**−**9**.

4. DISCUSSION

In a previous study, cumanin, helenalin, and hymenin derivatives were synthesized and evaluated against human tumor cell lines.[15] We have also demonstrated the trypanocidal activity of the STL cumanin against *T. cruzi*.[18] Based on these reports and taking into consideration that the synthesis of derivatives is a strategy used for the identification of more active and selective compounds, we evaluated the effect of the three natural STLs and their analogues on *T. cruzi*.

In our present work, we propose a new series of derivatives of hydroxyl groups present in the sesquiterpene lactones: acetyl, silyl, and triazolyl derivatives (related to imidazole ring in benznidazole) and the synthetic intermediates of the last, in the search of new active and selective trypanocidal drugs. Molecules also containing azole functions are of great interest and have shown antimicrobial activity.[26] Overall, silyl groups may not be considered only as protecting groups, but as a feasible strategy to introduce lipophilicity in drugs. On the other hand, acetate derivative fulfills a similar function.[27] The hydroxyl derivatives could allow us to evaluate the influence of these groups in the trypanocidal activity of the natural compounds.

The STLs cumanin, helenalin, and hymenin and their derivatives were initially tested on *T. cruzi* epimastigotes, a noninfective and replicative form of the parasite. Some of the evaluated compounds presented an IC_{50} in the range of benznidazole, indicating room for improvement. The *O*-alkylated derivative of cumanin (compound **7**) was the most active of the series derivatives obtained from cumanin with an IC_{50} = 1.27μg/mL (3.7 μM), followed by the diacetylated derivative, compound **2**, which presented an IC_{50} value similar to the natural compound cumanin [IC_{50} = 3.20 μg/mL (9.2 μM)]. The other derivatives were less active or inactive. In relation to helenalin (**10**) [IC_{50} = 0.29 μg/mL (1.11 μM)], its acetylated and silylated derivatives presented higher activity than the natural compound with IC_{50} values lower than 1 μM. Hymenin (**11**) and its silylated derivative (**15**) were active on *T. cruzi* epimastigotes with IC_{50} values of 1.50 μg/mL (5.8 μM) and 1.70 μg/mL (5.1 μM), respectively.

Although all of the tested compounds contain an α,β-unsaturated-γ-lactone moiety, differences in activity and selectivity have been registered. The increment in the trypanocidal activity of helenalin and hymenin and their derivatives was accompanied with cytotoxicity to mammalian cells. These compounds have an additional cyclopentenone group that could lead to an increase in toxicity.

Although the diacetylated derivative of cumin, showed similar activity to cumanin (**1**) on epimastigotes (IC_{50} = 2.98 μg/mL *vs* 3.20 μg/mL), it was 10 times more selective than the natural compound (**1**), highlighting the improvement of the diacetylation on this STL.

Helenalin and hymenin and their analogues were highly cytotoxic on mammalian cells, and they did not show selectivity of action (SI values lower than 1). Taking into account the trypanocidal activity and the selectivity of action presented by the diacetylated derivative of cumanin (**2**), this compound was selected for further evaluation on the trypomastigote and amastigote forms of the parasite. Although compound **7**, the propargyl ether derivative of cumanin, was also selective and more active than the natural compound

https://doi.org/10.1021/acsomega.1c05560
ACS Omega 2022, 7, 968−978

cumanin, the best yields obtained during the synthesis of the diacetylated derivative in comparison to compound **7** (75 *vs* 33%), determined that compound **2** was selected for further studies. In addition, the methodology for obtaining the acetylated derivative is simple for compounds that have secondary hydroxyl groups in their structure, since it involves few steps and reagents. Both cumanin and its diacetylated derivative showed moderate activity against *T. cruzi* trypomastigotes. However, these compounds were active against the intracellular form of the parasite, with cumanin diacetate being the most selective (SI = 52.4 *vs* 3.5 for cumanin).

Acetylation of active compounds has shown different effects: from obtaining a prodrug to a more active derivative by increasing the lipophilicity, which would improve the biodisponibility and reduce the toxicity of the drug. Regarding sesquiterpenoid compounds, Chen et al.[28] analyzed the effect of acetylation on halogenated sesquiterpenoids. The authors compared the cytotoxicity effects of the sesquiterpenoid elatol and the acetyl derivative on HeLa cells. The acetate analogue was 1-fold less toxic than the natural compound (IC_{50} 13.7 *vs* 1.3 μM, respectively). On the other hand, Harmatha et al.[29] demonstrated that the acetylation of the diol function present in the STL tribolide decreased the cytotoxicity on mammalian cells. The results found in relation to sesquiterpenes are in accordance with those obtained with cumanin and cumanin diacetate. Despite the trypanocidal activity did not increase, cytotoxicity on mammalian cells was reduced. Overall, these facts reinforce the hypothesis that acetylation plays an important role in decreasing the cytotoxicity of the corresponding alcohol.

There is a special interest in compounds that target the amastigote stage, particularly drugs that are active against the slow or nonproliferating forms of amastigotes as dormancy may play a key role in sterile cure.[30] In this sense, determination of its effect on dormant amastigotes as well as its combination with benznidazole or new vaccines as immunotherapies[31,32] will be an aspect of future research. The efficacy of cumanin diacetate in the *T. cruzi* K98 BALB/c infection model is in accordance with others reported for STLs[33−36] reinforcing the utility of this group of compounds *in vivo*. Even though sterile cure was not achieved, circulating parasites were significantly reduced. This fact is highly interesting considering the moderate activity of compounds evaluated *in vitro*. Even though selectivity of action is not improved compared with Bz, no weight loss was detected upon mice treatment with each drug. As the mechanism of action of cumanin diactetate might differ from that of benznidazole, this compound appears as an interesting lead to further improve its selectivity and to assess drug combination schemes with Bz. The high efficacy obtained *in vivo* might be related to drug metabolism and the activity in amastigotes.

The lack of sterile cure observed here might be related to the drug schedule that was employed as neither Bz was able to cure employing this regimen. Recently, extended treatment with Bz proved to be effective against dormant parasites, highlighting how playing with dosage and treatment length can improve drug efficacy.[37]

Notably, the reduction in parasitic load observed here was also associated with an improvement in animal well-being and tissue damage during the chronic phase of *T. cruzi* infection. We have previously demonstrated a similar outcome with other STLs like eupatoriopicrin.[38] Interestingly, apart from their trypanocidal activity, some STLs including the latter have shown anti-inflammatory properties.[39] Consequently, we can envision a scenario where STLs treatment might play a dual role reducing both parasitic load and immune-mediated damage at the same time. Although this remains to be proved for cumanin diacetate, further experiments will shed light on the matter.

Docking studies were performed to evaluate the possible interaction between cumanin and related compounds with TcTR, as a potential target. The docking experiment showed a correlation between the biological descriptor (IC_{50}), the hydrogen bonding capacity of the ligand, and the ΔG_{dock}, suggesting that it may be probable to find interactions between cumanin and its derivatives with TcTR. Other STLs such as deoxymikanolide and STLs of the 4,15-*iso*-atriplicolide type have affected or have shown inhibition of TcTR.[23,24]

The most promising STLs, compounds **2** and **7**, showed ΔG_{dock} in the range of komaroviquinona, a terpenoid compound that was taken as a reference for the study. It was found that the residues Glu466B, Ser470B, and Arg472B were involved in H-bonding of the most active STLs. Both compounds **2** and **7** showed interactions with the residues Arg472B and Ser470B. This latter residue, together with Glu467B, was involved in H-bonding with komaroviquinone. Moreover, the natural STL cumanin showed interactions with the three residues Arg472B, Ser470B, and Glu466B.

Further biological experiments will be needed to determine the inhibitory potency of the most promising STLs, compounds **2** and **7** on TcTR. Considering its essential role in the antioxidant machinery of the parasite, if these results are confirmed, they might explain the *in vivo* efficacy observed upon treatment with cumanin diacetate during the acute phase of infection.

It is important to point out that due to the high turnover of TcTR, high-affinity inhibitors are needed[40] so additional modification of cumanin diacetate that improves its binding might be an attractive area for future research in the development of novel drugs against *T. cruzi* infection.

5. CONCLUSIONS

The synthesis of derivatives has been a strategy for the optimization of natural compounds. The *in vitro* and *in vivo* activity of cumanin diacetate on *T. cruzi*, as well as the results of the docking study show the potential of this compound as a trypanocidal lead for the development of new drugs against Chagas disease.

AUTHOR INFORMATION

Corresponding Author

Valeria P. Sülsen − *Instituto de Química y Metabolismo del Fármaco (IQUIMEFA), CONICET—Universidad de Buenos Aires, 1113 Buenos Aires, Argentina; Cátedra de Farmacognosia, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, 1113 Buenos Aires, Argentina;* orcid.org/0000-0002-9322-5748; Phone: 54-11-5287-4286; Email: vsulsen@ffyb.uba.ar; Fax: 54-11-4508-3642

Authors

Andrés Sánchez Alberti − *Instituto de Microbiología y Parasitología Médica (IMPaM), CONICET—Universidad de Buenos Aires, 1121 Buenos Aires, Argentina; Cátedra de Inmunología, Facultad de Farmacia y Bioquímica,*

https://doi.org/10.1021/acsomega.1c05560
ACS Omega 2022, 7, 968−978

Universidad de Buenos Aires, 1113 Buenos Aires, Argentina; orcid.org/0000-0001-5369-2889

María F. Beer − *Facultad de Química, Bioquímica y Farmacia, Instituto de Investigaciones en Tecnología Química (INTEQUI-CONICET), Universidad Nacional de San Luis, Área de Química Orgánica, CP D5700HGC San Luis, Argentina; Instituto de Química y Metabolismo del Fármaco (IQUIMEFA), CONICET—Universidad de Buenos Aires, 1113 Buenos Aires, Argentina*

Natacha Cerny − *Instituto de Microbiología y Parasitología Médica (IMPaM), CONICET—Universidad de Buenos Aires, 1121 Buenos Aires, Argentina; Instituto de Estudios de la Inmunidad Humoral (IDEHU), CONICET—Universidad de Buenos Aires, 1113 Buenos Aires, Argentina*

Augusto E. Bivona − *Cátedra de Inmunología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, 1113 Buenos Aires, Argentina; Instituto de Estudios de la Inmunidad Humoral (IDEHU), CONICET—Universidad de Buenos Aires, 1113 Buenos Aires, Argentina*

Lucas Fabian − *Instituto de Química y Metabolismo del Fármaco (IQUIMEFA), CONICET—Universidad de Buenos Aires, 1113 Buenos Aires, Argentina*

Celina Morales − *Facultad de Medicina, Departamento de Patología, Instituto de Fisiopatología Cardiovascular, Universidad de Buenos Aires, Buenos Aires 1113, Argentina*

Albertina Moglioni − *Instituto de Química y Metabolismo del Fármaco (IQUIMEFA), CONICET—Universidad de Buenos Aires, 1113 Buenos Aires, Argentina*

Emilio L. Malchiodi − *Instituto de Microbiología y Parasitología Médica (IMPaM), CONICET—Universidad de Buenos Aires, 1121 Buenos Aires, Argentina; Cátedra de Inmunología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, 1113 Buenos Aires, Argentina; Instituto de Estudios de la Inmunidad Humoral (IDEHU), CONICET—Universidad de Buenos Aires, 1113 Buenos Aires, Argentina;* orcid.org/0000-0001-7501-3330

Osvaldo J. Donadel − *Facultad de Química, Bioquímica y Farmacia, Instituto de Investigaciones en Tecnología Química (INTEQUI-CONICET), Universidad Nacional de San Luis, Área de Química Orgánica, CP D5700HGC San Luis, Argentina*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c05560

Author Contributions

○A.S.A. and M.F.B contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Scientific and Technical Research Council (PIP 11220150100158CO and PIP 090), the National Agency for Science and Technology Promotion (PICT 2015−3531), the University of Buenos Aires (UBACYT 20020170100316BA), The National University of San Luis (Project PROICO 2-2620), and the Tricontinental Talent Program of the Canary Islands CEI: Tricontinental Atlantic Campus. This investigation is part of the activities within the "Research Network Natural Products against Neglected Diseases" (ResNet NPND): http://www.resnetnpnd.org/.

■ REFERENCES

(1) Sülsen, V. P.; Martino, V. Overview. In *Sesquiterpene Lactones. Advances in their Chemistry and Biological Aspects*, 1st ed.; Sülsen, V.; Martino, V., Eds.; Springer: Cham, Switzerland, 2018; pp 3−17.

(2) World Health Organization (WHO). Chagas Disease (American Trypanosomiasis). https://www.who.int/health-topics/chagas-disease#tab=tab_1 (Accessed July 29, 2021).

(3) Sülsen, V. P.; Puente, V.; Papademetrio, D.; Batlle, A.; Martino, V. S.; Frank, F. M.; Lombardo, M. E. Mode of Action of the Sesquiterpene Lactones Psilostachyin and Psilostachyin C on *Trypanosoma cruzi*. *PLoS One.* **2016**, *11*, No. e0150526.

(4) Berg, M.; Van der Veken, P.; Goeminne, A.; Haemers, A.; Augustyns, K. Inhibitors of the Purine Salvage Pathway: A Valuable Approach for Antiprotozoal Chemotherapy? *Curr. Med. Chem.* **2010**, *17*, 2456−2481.

(5) Steenkamp, D. J. Thiol metabolism of the trypanosomatids as potential drug targets. *IUBMB Life* **2002**, *53*, 243−248.

(6) Vázquez, K.; Paulino, M.; Salas, C. O.; Zarate-Ramos, J. J.; Vera, B.; Rivera, G. Trypanothione Reductase: A Target for the Development of Anti- Trypanosoma cruzi Drugs. *Mini-Rev. Med. Chem.* **2017**, *17*, 939−946.

(7) Caputto, M. E.; Fabian, L. E.; Benítez, D.; Merlino, A.; Ríos, N.; Cerecetto, H.; Moltrasio, G. Y.; Moglioni, A. G.; González, M.; Finkielsztein, L. M. Thiosemicarbazones derived from 1-indanones as new anti-*Trypanosoma cruzi* agents. *Bioorg. Med. Chem.* **2011**, *19*, 6818−6826.

(8) Burtoloso, A. C.; de Albuquerque, S.; Furber, M.; Gomes, J. C.; Gonçalez, C.; Kenny, P. W.; Leitão, A.; Montanari, C. A.; Quilles, J. C., Júnior; Ribeiro, J. F.; Rocha, J. R. Anti-trypanosomal activity of non-peptidic nitrile-based cysteine protease inhibitors. *PLoSP Neglected Trop. Dis.* **2017**, *11*, No. e0005343.

(9) San Francisco, J.; Barría, I.; Gutiérrez, B.; Neira, I.; Muñoz, C.; Sagua, H.; Araya, J. E.; Andrade, J. C.; Zailberger, A.; Catalán, A.; Remonsellez, F.; Vega, J. L.; González, J. Decreased cruzipain and gp85/trans-sialidase family protein expression contributes to loss of *Trypanosoma cruzi* trypomastigote virulence. *Microbes Infect.* **2017**, *19*, 55−61.

(10) Maya, J. D.; Cassels, B. K.; Iturriaga-Vásquez, P.; Ferreira, J.; Faúndez, M.; Galanti, N.; Ferreira, A.; Morello, A. Mode of action of natural and synthetic drugs against *Trypanosoma cruzi* and their interaction with the mammalian host. *Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol.* **2007**, *146*, 601−620.

(11) Benaim, G.; Hernandez-Rodriguez, V.; Mujica-Gonzalez, S.; Plaza-Rojas, L.; Silva, M. L.; Parra-Gimenez, N.; Garcia-Marchan, Y.; Paniz-Mondolfi, A.; Uzcanga, G. In vitro anti-*Trypanosoma cruzi* activity of dronedarone, a novel amiodarone derivative with an improved safety profile. *Antimicrob. Agents. Chemother.* **2012**, *56*, 3720−3725.

(12) Lombardo, M. E.; Batlle, A. Mode of action on Trypanosoma and Leishmania spp.. In *Sesquiterpene Lactones. Advances in their Chemistry and Biological Aspects*, 1st ed.; Sülsen, V.; Martino, V., Eds.; Springer: Cham, Switzerland, 2018; pp 223−240.

(13) Battista, T.; Colotti, G.; Ilari, A.; Fiorillo, A. Targeting Trypanothione Reductase, a Key Enzyme in the Redox Trypanosomatid Metabolism, to Develop New Drugs against Leishmaniasis and Trypanosomiases. *Molecules* **2020**, *25*, 1924.

(14) Saha, D.; Sharma, A. Docking-based screening of natural product database in quest for dual site inhibitors of *Trypanosoma cruzi* trypanothione reductase (TcTR). *Med. Chem. Res.* **2015**, *24*, 316−333.

(15) Beer, M. F.; Bivona, A.; Sánchez Alberti, A.; Cerny, N.; Reta, G.; Martín, V.; Padrón, J. M.; Malchiodi, E. L.; Sülsen, V. P.; Donadel, O. J. Preparation of Sesquiterpene Lactone Derivatives: Cytotoxic Activity and Selectivity of Action. *Molecules* **2019**, *24*, 1113.

(16) National Research Council (U.S.). *Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Guide for the Care and Use of Laboratory Animals*; National Academies Press: Washington D.C., 2011.

(17) Charan, J.; Kantharia, N. D. How to calculate sample size in animal studies? *J. Pharmacol. Pharmacother.* **2013**, *4*, 303−306.
(18) Sülsen, V. P.; Cazorla, S. I.; Frank, F. M.; Laurella, L. C.; Muschietti, L. V.; Catalán, C. A.; Martino, V. S.; Malchiodi, E. L. Natural terpenoids from Ambrosia species are active in vitro and in vivo against human pathogenic trypanosomatids. *PLoS Neglected Trop. Dis.* **2013**, *7*, No. e2494.
(19) Martin, D.; Postan, M.; Lucas, P.; Gress, R.; Tarleton, R. TGF-beta regulates pathology but not tissue CD8 +T cell dysfunction during experimental *Trypanosoma cruzi* infection. *Eur. J. Immunol.* **2007**, *37*, 2764−2771.
(20) Baell, J.; Holloway, G. New Substructure Filters for Removal of Pan Assay Interference Compounds (PAINS) from Screening Libraries and for Their Exclusion in Bioassays. *J. Med. Chem.* **2010**, *53*, 2719−2740.
(21) Sanchez Alberti, A.; Bivona, A. E.; Matos, M. N.; Cerny, N.; Schulze, K.; Weißmann, S.; Ebensen, T.; González, G.; Morales, C.; Cardoso, A. C.; Cazorla, S. I.; Guzmán, C. A.; Malchiodi, E. L. Mucosal Heterologous Prime/Boost Vaccination Induces Polyfunctional Systemic Immunity, Improving Protection Against *Trypanosoma cruzi*. *Front. Immunol.* **2020**, *11*, No. 128.
(22) Saeidnia, S.; Gohari, A. R.; Haddadi, A. Biogenic trypanocidal sesquiterpenes: lead compounds to design future trypanocidal drugs - a mini review. *Daru, J. Pharm. Sci.* **2013**, *21*, No. 35.
(23) Puente, V.; Laurella, L. C.; Spina, R. M.; Lozano, E.; Martino, V. S.; Sosa, M. A.; Sülsen, V. P.; Lombardo, E. Primary targets of the sesquiterpene lactone deoxymikanolide on *Trypanosoma cruzi*. *Phytomedicine* **2019**, *56*, 27−34.
(24) Lenz, M.; Krauth-Siegel, R. L.; Schmidt, T. J. Natural Sesquiterpene Lactones of the 4,15-*iso*-Atriplicolide Type are Inhibitors of Trypanothione Reductase. *Molecules* **2019**, *24*, No. 3737.
(25) Arnston, P. M.; Setzer, W. N. Macromolecular Targets of Antiparasitic Germacranolide Sesquiterpenoids: An *In Silico* Investigation. *Comb. Chem. High Throughput Screening* **2020**, *23*, 477−503.
(26) Reta, G. F.; Chiaramello, A. I.; García, C.; León, L. G.; Martín, V. S.; Padrón, J. M.; Tonn, C. E.; Donadel, O. J. Derivatives of grindelic acid: From a non-active natural diterpene to synthetic antitumor derivatives. *Eur. J. Med. Chem.* **2013**, *67*, 28−38.
(27) Padrón, J.; Donadel, O.; León, L.; Martín, T.; Martín, V. Enhancement of drug cytotoxicity by silicon containing groups. *Lett. Drug Des. Discov.* **2006**, *3*, 29−34.
(28) Chen, Q. F.; Liu, Z. P.; Wang, F. P. Natural sesquiterpenoids as cytotoxic anticancer agents. *Mini-Rev. Med. Chem.* **2011**, *11*, 1153−1164.
(29) Harmatha, J.; Vokáč, K.; Buděšínský, M.; Zídek, Z.; Kmoníčková, E. Immunobiological properties of sesquiterpene lactones obtained by chemically transformed structural modifications of trilobolide. *Fitoterapia* **2015**, *107*, 90−99.
(30) Sánchez-Valdéz, F. J.; Padilla, A.; Wang, W.; Orr, D.; Tarleton, R. L. Spontaneous dormancy protects *Trypanosoma cruzi* during extended drug exposure. *Elife* **2018**, *7*, No. e34039.
(31) Cerny, N.; Bivona, A. E.; Sanchez Alberti, A.; Trinitario, S. N.; Morales, C.; Cardoso Landaburu, A.; Cazorla, S. I.; Malchiodi, E. L. Cruzipain and Its Physiological Inhibitor, Chagasin, as a DNA-Based Therapeutic Vaccine Against *Trypanosoma cruzi*. *Front. Immunol.* **2020**, *11*, No. 565142.
(32) Sanchez Alberti, A.; Bivona, A. E.; Cerny, N.; Schulze, K.; Weißmann, S.; Ebensen, T.; Morales, C.; Padilla, A. M.; Cazorla, S. I.; Tarleton, R. L.; Guzmán, C. A.; Malchiodi, E. L. Engineered trivalent immunogen adjuvanted with a STING agonist confers protection against *Trypanosoma cruzi* infection. *NPJ Vaccines* **2017**, *2*, No. 9.
(33) Sülsen, V. P.; Frank, F. M.; Cazorla, S. I.; Anesini, C. A.; Malchiodi, E. L.; Freixa, B.; Vila, R.; Muschietti, L. V.; Martino, V. S. Trypanocidal and leishmanicidal activities of sesquiterpene lactones from Ambrosia tenuifolia Sprengel (Asteraceae). *Antimicrob Agents Chemother.* **2008**, *52*, 2415−2419.
(34) Sülsen, V. P.; Frank, F. M.; Cazorla, S. I.; Barrera, P.; Freixa, B.; Vila, R.; Sosa, M. A. M.; Malchiodi, E. L.; Muschietti, L. V.; Martino, V. S. Psilostachyin C: a natural compound with trypanocidal activity. *Int. J. Antimicrob. Agents* **2011**, *37*, 536−543.
(35) Branquinho, R. T.; de Mello, C. G. C.; Oliveira, M. T.; Reis, L. E. S.; Vieira, P. M. A.; Saúde-Guimarães, D. A.; Mosqueira, V. C. F.; de Lana, M. Lychnopholide in Poly(d,l-Lactide)-*Block*-Polyethylene Glycol Nanocapsules Cures Infection with a Drug-Resistant *Trypanosoma cruzi* Strain at Acute and Chronic Phases. *Antimicrob. Agents Chemother.* **2020**, *64*, No. e01937-19.
(36) Milagre, M. M.; Branquinho, R. T.; Gonçalves, M. F.; de Assis, G.; de Oliveira, M. T.; Reis, L.; Saúde-Guimarães, D. A.; de Lana, M. Activity of the sesquiterpene lactone goyazensolide against *Trypanosoma cruzi in vitro* and *in vivo*. *Parasitology* **2020**, *147*, 108−119.
(37) Bustamante, J. M.; Sanchez-Valdez, F.; Padilla, A. M.; White, B.; Wang, W.; Tarleton, R. L. A modified drug regimen clears active and dormant trypanosomes in mouse models of Chagas disease. *Sci. Transl. Med.* **2020**, *12*, No. eabb7656.
(38) Elso, O. G.; Bivona, A. E.; Sanchez Alberti, A.; Cerny, N.; Fabian, L.; Morales, C.; Catalán, C. A. N.; Malchiodi, E. L.; Cazorla, S. I.; Sülsen, V. P. Trypanocidal Activity of Four Sesquiterpene Lactones Isolated from Asteraceae Species. *Molecules* **2020**, *25*, 2014.
(39) Michalak, B.; Piwowarski, J. P.; Granica, S.; Waltenberger, B.; Atanasov, A. G.; Khan, S. Y.; Breuss, J. M.; Uhrin, P.; Żyżyńska-Granica, B.; Stojakowska, A.; Stuppner, H.; Kiss, A. K. Eupatoriopicrin Inhibits Pro-inflammatory Functions of Neutrophils via Suppression of IL-8 and TNF-alpha Production and p38 and ERK 1/2 MAP Kinases. *J. Nat. Prod.* **2019**, *82*, 375−385.
(40) Mendonça, A. A. S.; Coelho, C. M.; Veloso, M. P.; Caldas, I. S.; Gonçalves, R. V.; Teixeira, A. L.; de Miranda, A. S.; Novaes, R. D. Relevance of Trypanothione Reductase Inhibitors on *Trypanosoma cruzi* Infection: A Systematic Review, Meta-Analysis, and In Silico Integrated Approach. *Oxid. Med. Cell. Longevity* **2018**, *2018*, No. 8676578.

https://doi.org/10.1021/acsomega.1c05560
ACS Omega 2022, 7, 968−978