SUPPLEMENTAL MATERIAL

Material and Methods for *phenotypic screening*

Stock solutions: Stock compound solutions (30 mM) were prepared in DMSO (dimethyl sulphoxide) and fresh dilutions were prepared as needed, with the final concentration never exceeding 0.6% for *in vitro* experiments. The reference drug miltefosine was diluted in water at 20 mM.

Parasite strain and isolation: Amastigotes from *Leishmania amazonensis (LTB0016)* were purified from male BALBc at 30 days post infection. Briefly, the foot paws were inoculated (subcutaneously) with 20 µL containing 10^6 amastigotes, skin lesions were aseptically removed, and the parasite purified via mechanic dispersion in RPMI medium[1].

Mammalian cells and cytotoxic assays: Primary cultures of peritoneal macrophages (PMM) were obtained from Swiss male mice (18-20 g) previously inoculated with 1 mL 3% thioglycolate. After 96 hours of stimulation, the cells were harvested by rinsing the animals' peritoneum with RPMI 1-640. Peritoneal cells were then platted into 96-well microplates at a cell density of 10^5 cells per well, as reported[2]. The cultures were then sustained in RPMI 1-640 medium (pH 7.2 to 7.4) without phenol red (Gibco BRL) and supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine. After 24 hours, the compounds (up to 500 µM) were added for another 48 hours of incubation at 37º C. For colorimetric analysis, the treated cell cultures were incubated for 24 hours (5% CO_2 37ºC) with 10% AlamarBlue™ (Invitrogen) solution. The results were calculated according to the manufacturer's instructions and half maximum inhibitory concentrations (IC_{50}) were obtained to infer cytotoxicity[2].

Leishmanicidal susceptibility assays in vitro: Leishmanicidal whole cell-based screenings were performed against: (i) extracellular amastigotes forms (EF) collected from animal paw lesions, and on (ii) intracellular forms (IF) localized inside PMM infected for 2 h using amastigotes, and then, followed for another 48 h of infection. EFs assays (5×10^6 parasites/ mL in 96-well microplates, 5% CO_2 incubator) were performed at 32ºC by adding crescent concentrations of the studied compounds and incubating in RPMI 1-640 medium (pH 7.2 to 7.4) without phenol red (Gibco BRL) supplemented with 5% FBS, 1% L-glutamine, 1% penicillin-streptomycin, and the parasite load assessed by colorimetric analysis[3] in the presence of 10 % AlamarBlue™ (Invitrogen) solution. After 24 h, the absorbance was measured at 570 and 600 nm, following the manufacturer's instructions. In parallel, light microscopy analysis of untreated and drug-treated EF was done to quantify the number of live parasites. For IF assay, amastigotes purified from mice lesions were used to infect PMM (ratio 3:1) and sustained in RPMI 1-640 medium (pH 7.2 to 7.4) (Gibco BRL) supplemented with 10% FBS, 1% L-glutamine, 1% penicillin-Streptomycin at 37ºC, 5% CO_2 incubator. After 48 hours of drug exposure, samples were fixed with Bouin (5 min) and stained with Giemsa solution. The number of infected host cells (400 host cells scored) and parasites per infected cells were counted under a light microscope. Only characteristic parasite nuclei and kinetoplasts were counted as surviving parasites since irregular structures could mean parasites undergoing death. The drug activity was estimated by calculating the infection index (II - percentage of infected cells multiplied by the average number of intracellular parasites per infected host cell), and IC_{50} and IC_{90} values calculated by non-linear regression analysis using Graphpad Prism v.6. The assays were run at twice in triplicates.

In vivo evaluation in cutaneous leishmaniasis (CL) model: Male BALBc mice (18-20 g) were obtained from the animal facilities of ICTB (Institute of Science and Biomodels Technology/Fiocruz/RJ/Brazil) and housed 5 per cage (total of five groups), kept in a room at 20 to 24°C under a 12-h light and 12-h dark cycle, providing sterilized water and chow *ad libitum*. The animals were acclimated for 7 days before being inoculated (subcutaneously) on the left foot paw with 20 µL containing 5 x 10^5 amastigotes. The experimental design was previously standardized, with the treatment starting at the early lesion onset (15 days post infection - dpi), corresponding to lesion size diameter of 3-4 mm, assuring animal welfare during the 14 days of treatment (endpoint), while avoiding lesion ulceration. Mice were submitted to treatment using: Milteforan™ (Mt^{TM}), the reference drug, diluted in water for final doses of 4 (I) and 40 mg/kg (II) of body weight; compound **1** diluted in arabic gum vehicle 5% (6% DMSO, 3% Tween™80) dosed at 10 mg/kg of body weight, alone (III) or in co-administration with Mt^{TM} 4 mg/kg (IV), and arabic gum vehicle alone (V). All drug administrations were performed by oral gavage (po), once a day (qp) for Mt^{TM} and twice a day (bid) for **1**. Animals were monitored daily, and the lesion size regularly measured in 3 dimensions (height, width and depth). Euthanasia was carried out at 31º dpi, skin lesions were aseptically removed. Lesions were placed in a microtube filled with RNA *later* for qPCR quantification including 2 skin pieces from a non-infected mice foot paw, for PCR curves standardization[4]. The remaining lesion was used for imprint (fixed with methanol and stained in Giemsa) and light microscope observation.

Statistical analysis was conducted in Graphpad prism v.8.4.3 by ordinary one-way ANOVA Fisher's LSD test and by unpaired T test, significance $p < 0.05$ (95% confidence interval).

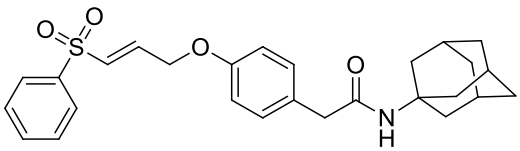
All procedures were carried out in accordance with the guidelines established by the FIOCRUZ Committee of Ethics for the Use of Animals (CEUA L038/2017).

Synthesis/Isolation and Characterization of Compounds 1-23

Compounds **1-21** were synthesized and characterized as reported by Zhang and co-workers[5]. Compounds **22 and 23** were isolated and characterized as reported by Carothers and co-workers[6]. The NMR (^{1}H and ^{13}C) and HRMS data for compound **1** is shown below and as Supplementary Fig 1 and 2.

Compound **1**

***N*-(adamantan-1-yl)-2-(4-(((*E*)-3-(phenylsulfonyl)allyl)oxy)phenyl)acetamide**



Eluent: hexane/ethyl acetate =1:2, RF = 0.4, white solid (35 %) ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (d, J = 7.5 Hz, 2H), 7.57 (t, J = 7.5 Hz, 1H), 7.48 (t, J = 7.5 Hz, 2H), 7.10 (d, J = 8.5 Hz, 2H), 6.61 (d, J = 8.5 Hz, 2H), 6.48 (d, J = 6.0 Hz, 1H), 5.08 (s, 1H), 4.90-4.86 (m, 1H), 4.05 (d, J = 8.0 Hz, 2H), 3.37 (s, 2H), 2.03 (s, 3H), 1.91 (s, 6H), 1.63 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.96, 155.50, 146.78, 138.60, 133.65, 130.96, 130.51, 128.96, 128.64, 117.01, 97.62, 52.63, 52.11, 44.00, 41.60, 36.36, 29.46. HRMS $[M+H]^+$ calculated for $C_{27}H_{31}NO_4S$: 466.2052; found: 466.2054.

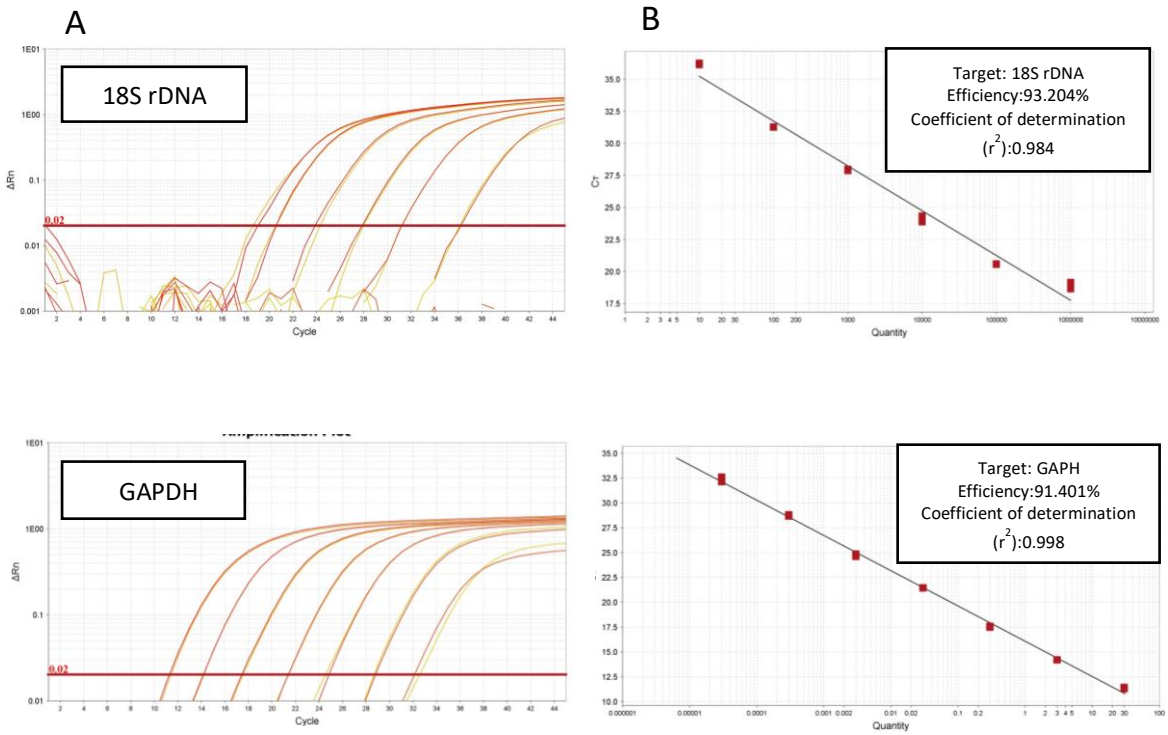


FIG S1 Real time quantitative PCR assays for parasite load quantitation from skin lesions of BALBc Infected with *Leishmania amazonensis*. The panel shows representative amplification plots with the fluorescent signal magnitude for *Leishmania* 18S rDNA and BALBc GAPDH targets (a) Amplification plots for *Leishmania* 18S rDNA (On the top) and BALBc GAPDH (On the bottom) targets indicate the dynamic extension of qPCR, (b) Standard curves for *Leishmania* 18S rDNA (On the top) and BALBc GAPDH (On the bottom) targets indicate the linearity of the qPCR assays, as well as the efficiency and coefficient of determination (r^2). qPCR assays were performed using TaqMan assays.

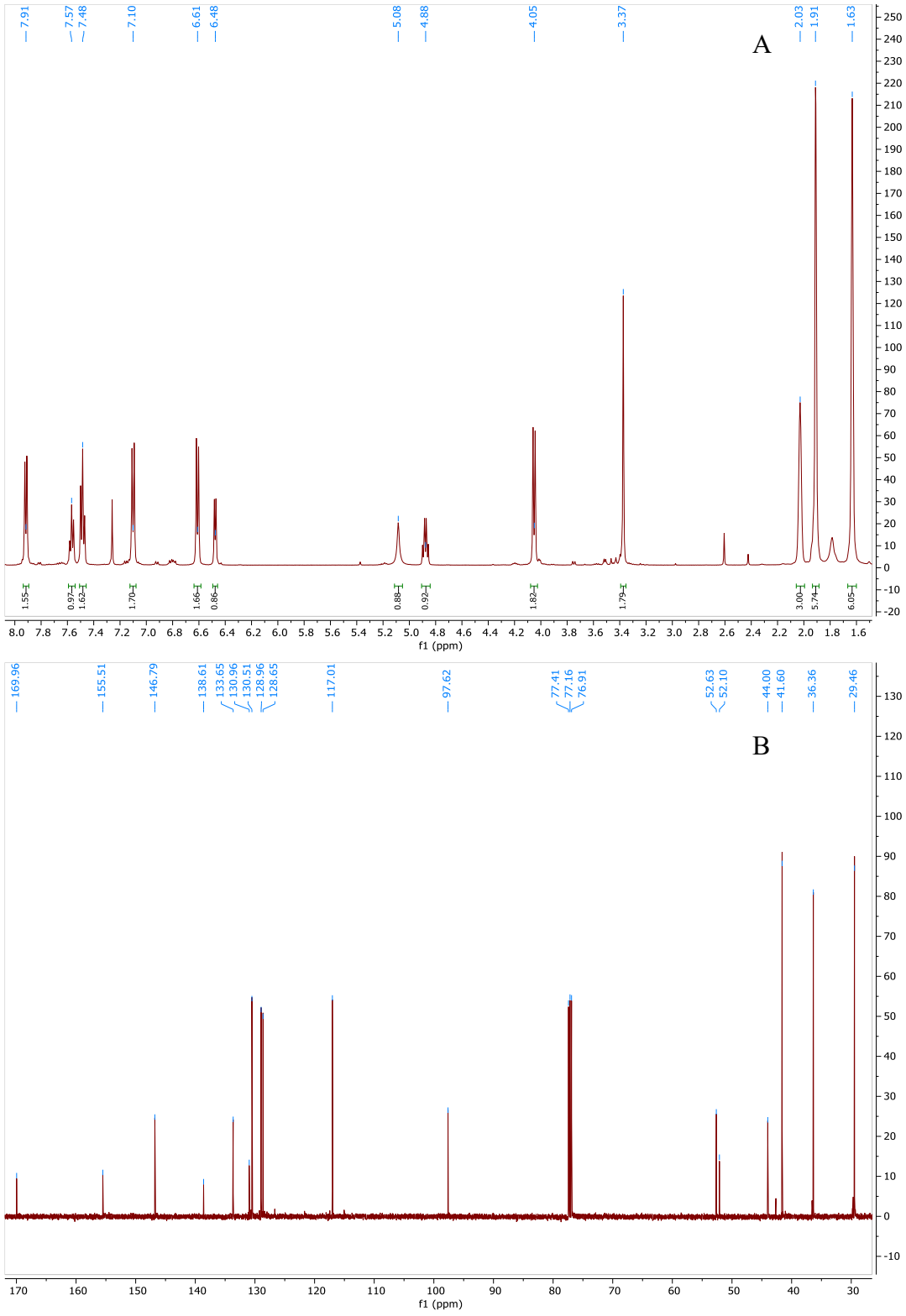


FIG S2 A: ^{1}H NMR Spectra of compound **1.** B: ^{13}C NMR spectrum of compound **1.**

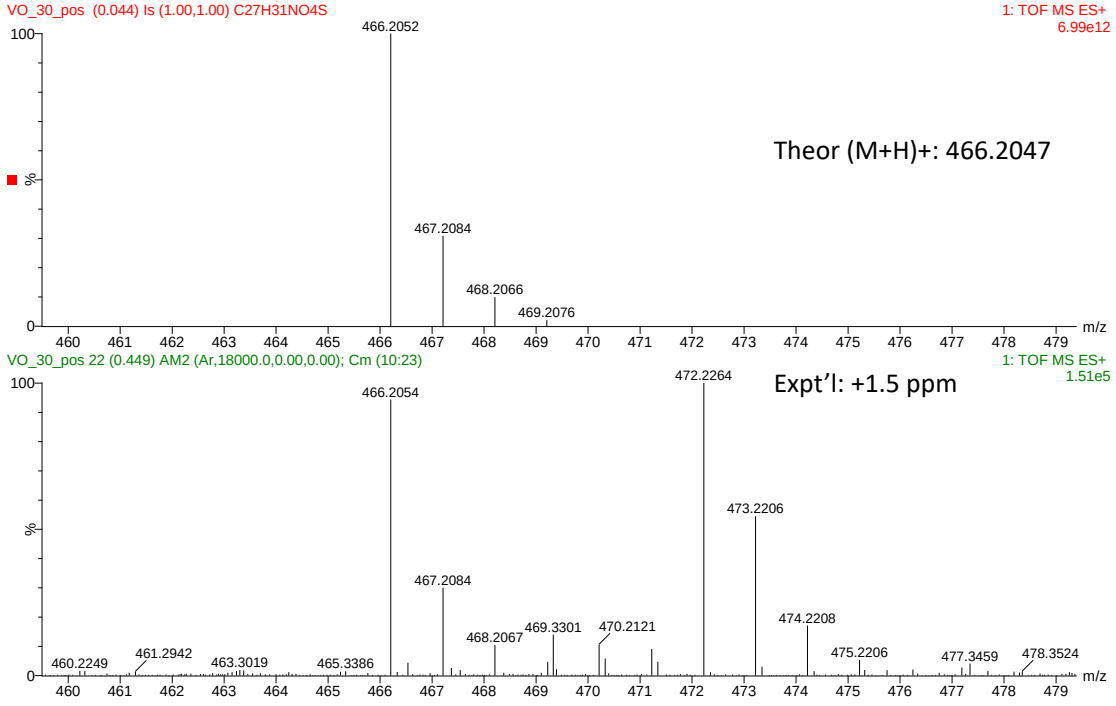


FIG S3 High resolution mass spectrum of compound **1**.

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