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In Silico Screening of Potential Drug with Antileishmanial Activity and Validation of their Activity by in Vitro and in Vivo Studies

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Abstract: The research on discovery and development of new treatments for cutaneous leishmaniasis has been declared as priority. Using bioinformatics approaches, this study aimed to identify antileishmanial activity in drugs that are currently used as anti-inflammatory and wound healing by such anti-*Leishmania* activity was validated by in vitro and in vivo assays. *In silico* analysis identified 153 compounds from which 87 were selected by data mining of DrugBank database, 22 and 44 were detected by PASS (www.way2drug.com/passonline) and BLAST (http://blast.ncbi.nlm.nih. gov/) alignment, respectively. The majority of identified drugs are used as skin protector, anti-acne, anti-ulcerative (wound healer) or anti-inflammatory and few of them had specific antileishmanial activity. The efficacy as antileishmanial was validated in vitro in 12/23 tested compounds and in all seven compounds that were evaluated in in vivo assays. Notably, this is the first report of antileishmanial activity for adapalene. In conclusion, bioinformatics tools not only can help to reduce time and cost of the drug discovery process but also may increase the chance that candidates identified *in silico* which have a validated antileishmanial activity by combining different biological properties.

Key words: Bioinformatic screening, blast, second uses, antileishmanial activity, leishmaniasis.

1. Introduction

Leishmaniasis is disease resulted after infection with protozoan *Leishmania* parasites. This disease is manifested as ulcers into the skin or mucouses, named as CL (cutaneous) and ML (mucosal leishmaniasis), respectively. A more severe infection known as VL (visceral leishmaniasis) is manifested with damage of vital organs and tissues (liver, spleen and bone marrow) [1]. The disease is spread worldwide being endemic in 99 countries where more than 350 million people are at risk of acquiring infection and 12 million people are infected. Despite the high number of clinical cases, only one offour cases is diagnosed in Latin America [2]. The pentavalent antimonial MA (meglumine antimoniate) and sodium stibogluconate, pentamidine isethionate and miltefosine are the drugs of choice for treatment of CL. Although they are still effective, these drugs have significant drawbacks, including systemic toxicity which is associated with high doses and prolonged treatment regimens causing abandon of treatment that affects drug efficacy [1]. Thereof, WHO (World Health Organization) has declared as priority the research on discovery and development of new treatments that would be more accessible, efficient, safer, easily to administer and at reasonable cost to improve the quality of life of patients [3].

Currently, bioinformatic is a strategy that may accelerate discovery of new drug saving experimental resources, especially in terms of in vitro and in vivo assays [4]. Thousands of proteins with different biological and pharmaceutical properties such as molecular targets [5], inhibitors [6], second uses [7] and target-ligand or target-drug interactions [8] registered in databases can be analyzed by

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bioinformatic tools, either individually or simultaneously. Thus, the use of virtual screening of millions of compounds on protein structure targets allows the selection of the most promising drug candidates [9-11]. Furthermore, the known proteome of three *Leishmania* species [12] have allowed the construction of complex interaction networks named interactomes [13] which could be useful in prediction of essential proteins that can be used as targets for the identification of drugs acting on those essential proteins [14, 15]. The identified molecules (drugs) can then be evaluated in vitro and in vivo systems using the appropriated model of disease or clinical condition. Considering that in CL, an effective drug should not only be able to kill *Leishmania* parasite but also regulate immune factors that participate in the resolution of the infection stimulating wound healing, using bioinformatic tools this study aimed to identify antileishmanial compound by combining different pharmacological properties in registered drugs. The validation of antileishmanial potential was validated b*y* in vitro and in vivo assays.

2. Experimental Methods

2.1 In Silico Identification of Drugs with Potential Antileishmanial Activity

DrugBank data base (www.drugbak.ka) that contains information about 7,759 approved and experimental drugs was filtered by data mining to identify drugs used as anti-inflammatory, or to treat ulcers and other skin conditions. The structures of the selected compounds in DrugBank were compared using the software PASS (www.way2drug.com/passonline) [16] against anti-protozoal compounds and those most similar is selected. The cutoff was set as the score given for drugs commonly used in treatment of leishmaniasis and other diseases caused by protozoan parasites such as *Plasmodium*, *Trypanosoma cruzi* and *Toxoplasma gondii.* In addition, an alignment of the *Leishamnia* proteins with the DrugBank targets was performed using BLAST (http://blast.ncbi.nlm.nih. gov/) [16]; then, the targets that showed the highest similarity with *Leishmania* proteins were selected and a list of the corresponding targets of selected compounds was obtained. Compounds with potential anti-*Leishmania* activity were selected according to an affinity cutoff higher than 0.8.

2.2 Cell Lines

Human U-937 promonocytes (CRL1593.2™) and HepG2 hepatocytes (HB-8065™) were obtained from the ATCC® (American Type Culture Collection Manassas, VA, USA) and cultured in standard conditions at 37 ℃, 5% CO_2, with change of medium every three days until use. U-937 cells were cultured in RPMI (Roswell Park Memorial Institute) 1640 (Sigma-Aldrich, St Louis MO, USA) with 10% FBS (fetal bovine serum) (Gibco, Life technologies Gaithersburg MD, USA) and 1% antibiotics (10,000 units penicillin and 10 mg/mL streptomycin) (Sigma-Aldrich). HepG2 cells were maintained in DMEM (Dulbecco's modified eagle medium) (Sigma-Aldrich) with 5% FBS and 1% antibiotics. In turn, macrophages of hamsters were derived from peritoneal mononuclear cells from three healthy donors (previously stimulated with 0.4% thyoglycolate). Cells were isolated from EDTA (ethylene diamine tetra aceticacid) anticoagulated exudated using Ficoll-Hypaque 1.077 (Sigma-Aldrich) according to manufacturer's instruction. After lysis of red blood cells with water and 3.6% sodium chlorate solution and centrifugation, supernatant was discarded. Mononuclear cells were counted and resuspended in RPMI 1640 supplemented with 10% FBS and 1% antibiotics at 5×10^5 cells/mL. One hundred μL were dispensed into each well of 96-well culture cell plate and incubated at 37 ℃, 5% CO_2 during 24 h to allow adherence and transformation into macrophages [17].

2.3 Parasites

Leishmania (V) panamensis transfected with the GFP (green fluorescent protein) (MHOM/CO/87/UA140-pIR-eGFP) was used.

Parasites were cultured as promastigotes at 26 ℃ in biphasic medium which consist in a solid phase of modified NNN (Novy-MacNeal-Nicolle) medium and a liquid phase of PBS (phosphate buffer saline) plus glucose, pH 6.9. In turn, intracellular amastigotes were obtained after infection of U-937 cells with promastigotes as follows: U-937 cells were dispensed in 24-well plates at 300,000 cells/well and treated with 1.0 µM of PMA (phorbol myristate acetate) (Sigma-Aldrich) for 48 h at 37 ℃. Then, cells were infected with promastigotes in stationary growth phase (day 5) at a ratio of 30:1 promastigotes/cell and incubated 3 h at 34 ℃ in 5% CO_2. Cells were washed twice with PBS to eliminate extracellular (free) parasites and 1.0 mL fresh RPMI-1640 was added into each well; plates were incubated again at 34 ℃ and 5% CO_2 to allow intracellular differentiation to amastigotes. After 24 h of infection, cells were ready to use in antileishmanial assays as described below (shown in 2.6 section).

2.4 Compounds

Adapalene, azelaic acid, Salicylhydroxamic acid, Docosanol, Alendronate, Phenylbutazone, Propantheline, Eucalyptol, Nepafenac, T198765, Fludrocortisone, Bepridil, Pranlukast, Imatinib and Amphotericin B were acquired from Sigma-Aldrich. Homatropine Methylbromide, Bentoquantam, Diclofenac, Dapsone, Carbenoxolone, Pantoprazole, Pamidronate and Primaquine were purchased in Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). MA was purchased from Sanofi-Aventis (Bogota, Colombia). Marimasmat was obtained from Calbiochem (Merck Millipore Corporation, Darmstadt, Germany).

2.5 In Vitro Cytotoxicity Assay

Cytotoxicity of compounds was determined in mammalian U-937 and HepG2 cells according to the effect on the cell growth determined by MTT microenzimatic method, as described by others [18]. In turn, cytotoxicity in haPM was assessed using alamarBlueR assay (Thermo Scientific Waltham, MA, USA) as described elsewhere [19]. Briefly, 2.0×10^4 U-937 or 2.5×10^4 HepG2 cells in 200 µL corresponding culture medium were dispensed into each well of 96-well tissue culture plate. Then, 100 µL/well of each compound at the corresponding dilution (200, 100, 50, 25, 12.5 and 6.25 µL/mL) were added and plates were incubated at 37 ℃, 5% CO_2.

In MTT assay, after 72 h of incubation 20 µL of MTT (Sigma-Aldrich) were added to each well and plates were incubated at 37 ℃, 5% CO_2 during 3h. Reaction was stopped by adding 100 µL/well of 50% isopropanol solution with 10% sodium dodecyl sulfate and 30 min incubation. The concentration of formazan was determined at 570 nm in a spectrophotometer (Varioskan Flash Multimode Reader, Thermo Scientific) and the intensity of color was registered as O.D (optical densities). In turn, in alamarBlueR assay, after 72 h of incubation plates were centrifuged, supernatant was discarded and, 100 µL of 1/10 alamar Blue-RPMI 1640 mixture (v/v) was added into each well. Plates were incubated 90 minutos at 37 ℃, 5% CO_2 and dark. The intensity of fluorescence was detectes at 530 nm excitation and 590 nm emission in a spectrophotometer (Varioskan Flash Multimode Reader) and the intensity of fluorescence was registered as R.F.U (relative fluorescence unit).

Cells treated with amphotericin B (standard antileishmanial drug) and doxorubicin were used as control for cytotoxicity (positive control) while cell incubated in absence of any compound or drug were used as control for growth cell (negative control). Determinations were done by triplicate in at least two independent experiments.

Cytotoxicity was determined according to the percentages of viability and cell growth inhibition obtained for each compound, amphotericin B, doxorubicin or medium alone. Percentages of viability were calculated using Eq. (1), as follows: % viability = (O.D. of treated cells)/(O.D. of control cells) × 100,

where the O.D. of the control cells corresponds to 100% viability. In turn, the percentage of cell growth inhibition is calculated using the Eq. (2) as follows: % cell growth inhibition = 100 % viability. The results are expressed as LC_{50} (lethal concentration 50). That corresponds to the concentration of drug that gives the half-maximal inhibition of the cell growth. The LC_{50} was calculated by the Probit method [20] and degree of cytotoxicity of each product was graded according to the LC_{50} values, using the own scale: Highly cytotoxicity: LC_{50} < 50 μg/mL; Moderate cytotoxicity LC_{50} > 50 to < 200 μg/mL and potential non cytotoxicity: LC_{50} > 200 μg/mL.

2.6 In Vitro Antileishmanial Activity

The effect of compounds against intracellular amastigotes of *L. panamensis* was evaluated by flow cytometry using the methodology described [21, 22]. After 24 h of infection of U-937 cells, culture medium was replaced by fresh RPMI-1640 medium containing each compound at any of four serial dilution base four (starting at a concentration not exceeding the LC_{50} determined previously). Infected and treated cells were maintained at 34 ℃, 5% CO_2. After 72h cells were removed from the bottom plate with a trypsin/EDTA (ethylenediaminetetraacetic-acid disodium salt) (250 mg) solution and centrifuged at 1.100 rpm, 10 min at 4 ℃; the supernatant was discarded and cells were washed with 1 mL of cold PBS and centrifuged again at 1.100 rpm, 10 min at 4 ℃, supernatant was discarded and cells were suspended in 500 μL of cold PBS. Cells were analyzed in an argon laser flow cytometer (Cytomics FC 500 MPL Beckman Coulter, Brea, CA, USA) reading at 488 nm of excitation and 525 nm of emission. Infected cells were determined according the positive events for green fluorescence (parasites).

Infected cells exposed to amphotericin B and MA were used as control of antileishmanial activity (positive control) while infected cells incubated in culture RPMI-1640 medium alone were used as control of infection (negative control). Each concentration was tested in triplicate in two independent experiments. Antileishmanial activity was determined according to reduction (inhibition) of parasites in each experimental condition calculated according to the Eq. (3) as follows: % infection = (% infected and treated cells/% infected and untreated cells) × 100. In turn, the percentage of inhibition was calculated using Eq. (4), as follows: % inhibition = 100 − % infection. The antileishmanial activity was expressed as the EC_{50} calculated by the Probit method as described above [20]. The EC_{50} corresponds to the concentration of drug that gives the half-maximal inhibition of the intracellular parasites.

The degree of antileishmanial activity was established as convenience according to the EC_{50} values, using the following our own scale: activity: EC_{50} < 20 μg/mL, moderate activity: EC_{50} > 20 to < 70 μg/mL; and potential non activity: EC_{50} > 50 μg/mL. The TI (therapeutic index) or SI (selectivity index) was calculated by dividing the cytotoxicity and the antileishmanial activity, using the Eq. (5): TI = CL_{50}/CE_{50}.

2.7 In Vivo Leishmanicidal Response

The most in vitro active compounds were then tested in vivo to evaluate their therapeutical response in the hamster (*Mesocricetus auratus*) model for CL [23]. Briefly, previously anesthetized (ketamine 40 mg/kg and xylazine 5 mg/kg) hamsters were inoculated in the dorsal skin with promastigotes of *L. panamensis* (5×10^8 parasites/100 μL PBS). Twelve experimental groups (n = 5 each) consisting of males and females, were formed. The compound and concentration tested were: adapalene 1% (group A), alendronate 4% (group B), alendronate 10 mg/kg/day (group C), azelaic acid 4% (group D); bentoquantam 5% (group E); bepridil 2.3% (group F); propanteline bromide 0.5% (group G); salicylhydroxamicacid 4% (group H) and MA, 120 mg/kg/day (group I). Doses were selected as convenience.

Treatments were initiated immediately after development of a typical ulcer (4-6 weeks post infection). Treatment were administered topically (40 mg per dose), orally (20 μL per dose) or intramuscularly (100 μL per dose) once every day during two weeks with exception of MA that was administered during 10 days. Animal welfare was supervised daily during the study. Areas of the ulcer and body weight were measured every two weeks from the beginning of treatments to the end of the study (three months after completion of treatment). The overall time points of evaluation were: pretreatment day (D0), end of treatment (D14) and post treatment days 30, 60 and 90 (PTD30, PTD60 and PTD90, respectively). At the end of the study, hamsters were humanely sacrificed and after necropsy, liver and kidney biopsies were taken for histopathological studies. A sample of the ulcer was also taken to determine parasite load by limiting dilution as described below.

The effectiveness of each treatment was assessed comparing the lesion sizes prior to and after treatments. Treatment outcome at the end of study was recorded as *cure* (healing of 100% area and complete disappearance of the lesion); *improvement* (reducing the size of the lesion in > 30% of the area); *failure* (increasing the size of the lesion) or *relapse* (reactivation of lesion after initial cure). To compare the effectiveness among groups of treatments an arbitrary score was assigned to each treatment outcome: 3 = cure, 2 = improvement, 1 = relapse and 0 = failure.

The toxicity of treatments was evaluated by comparingthe blood levels of ALT (alanine amino transferase), BUN (blood urea nitrogen) and creatinine using commercially available kits (Biosystems, Spain) as described by others [24]. At days D0 and day 8 of treatment (D8), blood was drawn from the hearth and serum was separated by centrifugation at 5,000 g for 2-3 min. The serum was stored at -80 ℃ until use. Toxicity of treatments was also determined by post-mortem necropsy and histological changes in liver and kidney. Severity of histological changes was also graded as severe, moderate or mild. Lastly, the number of living *L. panamensis* parasites in infected tissues was determined by serial dilutions of the skin homogenates incubated at 26 ℃. After 10-14 days, plates were read microscopically and the number of viable parasites was determined as described [25].

2.8 Ethical Aspects

The Ethics Committee for Animal Research of the University of Antioquia approved all procedures involving the uses and care of animals (Act 65, 2010).

3. Results and Discussion

In silico analysis identified 153 compounds from which 87 were selected by data mining of DrugBank database, 22 were detected by PASS analysis and 44 were detected by BLAST analysis of *Leishmania* gene targeting (Fig. 1). Among the 87 drugs from DrugBank four are used as skin protector, two are anti-acne, 17 are anti-ulcerative or wound healer and 64 are anti-inflammatory (Table 1, supplementary material). On the other hand, among 22 compounds identified by prediction of the antiparasite activity based on PASS structure, four of them had specific antileishmanial activity and 18 with activity against other protozoan parasites such as *Trypanosoma*, *Toxoplasma*, *Plasmodium*, coocidia, *Thrichomona*, *Histomona*, *Babesia*, and amoebas (Table 2 supplementary material). Finally, among 44 compounds identified as potential inhibitors of proteins (hypothetic valor confirmed) previously identified in *L. major*, *L. infantum* or *L. braziliensis* genome, five compounds are used as antibacterial, six are anti-inflammatory, four are antiprozoal, four are antitumor or anti-neoplasic and 14 compounds are used as anti-viral, anti-fungal, anti-anginal or anti-hypertensive, bone anti-resorption and others. The last 11 compounds are still in experimental phases (Table 3, supplementary material).

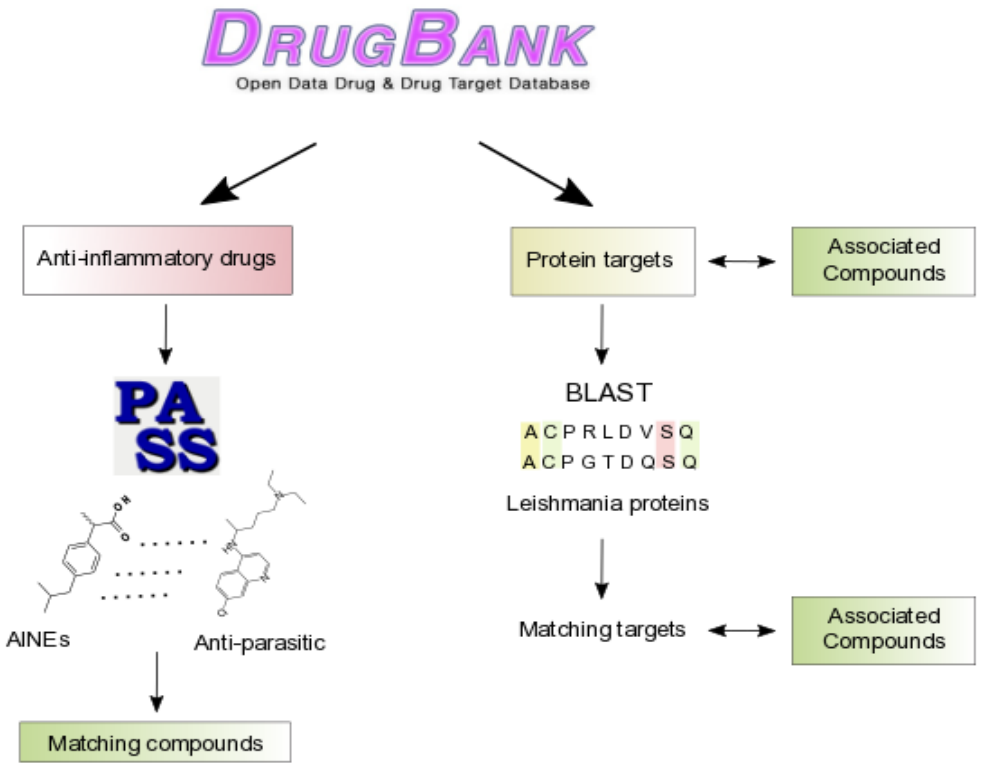

Fig. 1 ***In silico*** **strategy. DrugBank data base was filtered by data mining to identify drugs used as anti-inflammatory, or to treat ulcers and other skin conditions. Then, the structures of the selected compounds in DrugBank were compared using the software PASS against anti-protozoal compounds and those most similar was selected. Finally, an alignment of the** ***Leishamnia*** **proteins with the DrugBank targets was performed using BLAST. Dotted lines represent reference values.**

Table 1 List of drugs approved as skin protector, treatment of skin problems, anti-ulcerative/wound healing or anti-inflammatory activities detected *in silico*.

DrugBank ID	Name	Target
Skin protector		
DB00516	Bentoquatam	Not reported in DrugBank
DB00840	Hydroxypropyl cellulose	Not reported in DrugBank
DB00982	Isotretinoin	Retinoic acid receptor alpha, P10276
DB01216	Finasteride	3-oxo-5-alpha-steroid 4-dehydrogenase 2, P31213 3-oxo-5-alpha-steroid 4-dehydrogenase 1, P18405 3-oxo-5-beta-steroid 4-dehydrogenase, P51857
Skin lesions (anti-acne, anti-ulcerative/wound healing)		
DB00982	Isotretinoin	Retinoic acid receptor alpha, P10276
DB00162	Vitamin A	Retinol dehydrogenase 12, Q96NR8
DB00364	Sucralfate	Fibroblast growth factor 2, P09038 Pro-epidermal growth factor, P01133 Fibrinogen alpha chain, P02671 Fibrinogen beta chain, P02675 Fibrinogen gamma chain P02679 Serum albumin, P02768
DB02329	Carbenoxolone	3-alpha-(or20-beta)-hydroxysteroid dehydrogenase, P19992 Corticosteroid 11-beta-dehydrogenase isozyme 1, P28845
DB00048	Collagenase	Collagen alpha-1(I) chain, P02452 Collagen alpha-1(II) chain, P02458 Collagen alpha-1(III) chain, P02461 Collagen alpha-2(I) chain, P08123

Table 1 to be continued

B00102	Becaplermin	Platelet-derived growth factor receptor beta, P09619 Platelet-derived growth factor receptor alpha, P16234 Alpha-2-macroglobulin, P01023
DB01014	Balsalazide	Peroxisome proliferator-activated receptor gamma, P37231 Prostaglandin G/H synthase 2, P35354 Prostaglandin G/H synthase 1, P23219 Arachidonate 5-lipoxygenase, P09917 NADPH azoreductase, Q9FAW5
DB00585	Nizatidine	Histamine H2 receptor, P25021
DB00725	Homatropine methylbromide	Muscarinic acetylcholine receptor M2, P08172 Muscarinic acetylcholine receptor M1, P11229 Muscarinic acetylcholine receptor M4, P08173 Muscarinic acetylcholine receptor M5, P08912 Muscarinic acetylcholine receptor M3, P20309
DB00863	Ranitidine	Histamine H2 receptor, P25021
DB00927	Famotidine	Histamine H2 receptor, P25021
DB01129	Rabeprazole	Potassium-transporting ATPase alpha chain 1, P20648
DB03467	Naringenin	HTH-type transcriptional regulator TtgR, Q9AIU0
DB08806	Roxatidine acetate	Histamine H2 receptor, P25021
DB00213	Pantoprazole	Potassium-transporting ATPase alpha chain 1, P20648
DB00338	Omeprazole	Potassium-transporting ATPase alpha chain 1, P20648
DB00670	Pirenzepine	Muscarinic acetylcholine receptor M1, P11229
DB00782	Propantheline bromuro	Muscarinic acetylcholine receptor M1, P11229
DB00448	Lansoprazole	Potassium-transporting ATPase alpha chain 1, P20648
Anti-inflammatory		
DB00741	Hydrocortisone	Glucocorticoid receptor, P04150 Annexin A1, P04083
DB01260	Desonide	Glucocorticoid receptor, P04150
DB02266	Flufenamic Acid	Prostaglandin G/H synthase 2, P35354 Prostaglandin G/H synthase 1, P23219 Aldo-keto reductase family 1 member C3, P42330 Androgen receptor, P10275
DB02478	9alpha-Fluorocortisol	Mineralocorticoid receptor, P08235 Glucocorticoid receptor, P04150 Androgen receptor, P10275
DB04652	(11-beta)-11,21-dihydroxy-pregn-4-en e-3,20-dione	Mineralocorticoid receptor, P08235 Corticosteroid 11-beta-dehydrogenase isozyme 1, P28845 Nuclear receptor coactivator 1, Q15788
DB00812	Phenylbutazone	Prostaglandin G/H synthase 2, P35354 Prostacyclin synthase, Q16647 Prostaglandin G/H synthase 1, P23219
DB00991	Oxaprozin	Prostaglandin G/H synthase 1, P23219 Prostaglandin G/H synthase 2, P35354
DB01130	Prednicarbate	Glucocorticoid receptor, P04150
DB00223	Diflorasone	Glucocorticoid receptor, P04150
DB00253	Medrysone	Glucocorticoid receptor, P04150
DB00547	Desoximetasone	Glucocorticoid receptor, P04150
DB00846	Flurandrenolide	Glucocorticoid receptor, P04150
DB00896	Rimexolone	Glucocorticoid receptor, P04150
DB01380	Cortisone acetate	Glucocorticoid receptor, P04150
DB01384	Paramethasone	Glucocorticoid receptor, P04150

Table 1 to be continued

DB00591	Fluocinolone Acetonide	Glucocorticoid receptor, P04150
DB00596	Halobetasol Propionate	Glucocorticoid receptor, P04150
DB00620	Triamcinolone	Glucocorticoid receptor, P04150
DB00663	Flumethasone Pivalate	Glucocorticoid receptor, P04150
DB00687	Fludrocortisone	Mineralocorticoid receptor, P08235 Glucocorticoid receptor, P04150 Androgen receptor, P10275
DB01013	Clobetasol	Glucocorticoid receptor, P04150
DB01398	Salicylate-sodium	Prostaglandin G/H synthase 2, P35354 Prostaglandin G/H synthase 1, P23219
DB06725	Lornoxicam	Prostaglandin G/H synthase 1, P23219 Prostaglandin G/H synthase 2, P35354
DB06781	Difluprednate	Glucocorticoid receptor, P04150
DB00580	Valdecoxib	Prostaglandin G/H synthase 2, P35354
DB06802	Nepafenac	Prostaglandin G/H synthase 1, P23219 Prostaglandin G/H synthase 2, P35354
DB00180	Flunisolide	Glucocorticoid receptor, P04150
DB00210	Adapalene	Retinoic acid receptor gamma, P13631 Retinoic acid receptor beta, P10826 Retinoic acid receptor beta, P10826 Retinoic acid receptor RXR-beta, P28702 Retinoic acid receptor RXR-beta, P28702 Retinoic acid receptor RXR-alpha, P19793
DB00240	Alclometasone	Glucocorticoid receptor, P04150
DB00324	Fluorometholone	Glucocorticoid receptor, P04150
DB00586	Diclofenac	Prostaglandin G/H synthase 2, P35354 Prostaglandin G/H synthase 1, P23219 Arachidonate 5-lipoxygenase, P09917 Sodium channel protein type 4 subunit alpha, P35499 Acid-sensing ion channel 1, P78348 Potassium voltage-gated channel subfamily KQT member 2, O43526 Potassium voltage-gated channel subfamily KQT member 3, O43525 Phospholipase A2, membrane associated, P14555
DB00764	Mometasone	Glucocorticoid receptor, P04150
DB00769	Hydrocortamate	Glucocorticoid receptor, P04150
DB00873	Loteprednol	Glucocorticoid receptor, P04150
DB00959	Methylprednisolone	Glucocorticoid receptor, P04150
DB01047	Fluocinonide	Glucocorticoid receptor, P04150 Smoothened homolog, Q99835
DB01222	Budesonide	Glucocorticoid receptor, P04150
DB03852	Eucalyptol	Not reported in DrugBank
DB00328	Indomethacin	Prostaglandin G/H synthase 1, P23219 Prostaglandin G/H synthase 2, P35354 Phospholipase A2, membrane associated, P14555 Prostaglandin reductase 2, Q8N8N7 Peroxisome proliferator-activated receptor gamma, P37231 Lactoylglutathione lyase, Q04760 Prostaglandin D2 receptor 2, Q9Y5Y4 Peroxisome proliferator-activated receptor alpha, Q07869
DB00443	Betamethasone	Glucocorticoid receptor, P04150

Table 1 to be continued

DB00605	Sulindac	Prostaglandin G/H synthase 2, P35354 Prostaglandin G/H synthase 1, P23219 Aldose reductase, P15121 Mitogen-activated protein kinase 3, P27361 Peroxisome proliferator-activated receptor delta, Q03181 Prostaglandin D2 receptor 2, Q9Y5Y4
DB00712	Flurbiprofen	Prostaglandin G/H synthase 1, P23219 Prostaglandin G/H synthase 2, P35354
DB00749	Etodolac	Prostaglandin G/H synthase 2, P35354 Prostaglandin G/H synthase 1, P23219 Retinoic acid receptor RXR-alpha, P19793
DB00861	Diflunisal	Prostaglandin G/H synthase 2, P35354 Prostaglandin G/H synthase 1, P23219
DB01009	Ketoprofen	Prostaglandin G/H synthase 1, P23219 Prostaglandin G/H synthase 2, P35354 C-X-C chemokine receptor type 1, P25024
DB01050	Ibuprofen	Prostaglandin G/H synthase 2, P35354 Prostaglandin G/H synthase 1, P23219 Apoptosis regulator Bcl-2, P10415 Thrombomodulin P07204 Tissue-type plasminogen activator P00750 Fatty acid-binding protein, intestinal P12104 Peroxisome proliferator-activated receptor gamma P37231 Cystic fibrosis transmembrane conductance regulator P13569
DB00152	Thiamine	Thiamin pyrophosphokinase 1, Q9H3S4 Thiamine transporter 1, O60779
DB00165	Pyridoxine	Pyridoxal kinase O00764
DB00216	Eletriptan	5-hydroxytryptamine receptor 1D, P28221 5-hydroxytryptamine receptor 1B, P28222 5-hydroxytryptamine receptor 1F, P30939 5-hydroxytryptamine receptor 1A, P08908 5-hydroxytryptamine receptor 1E, P28566 5-hydroxytryptamine receptor 2B, P41595 5-hydroxytryptamine receptor 7, P34969
DB00282	Pamidronate	Farnesyl pyrophosphate synthase, P14324
DB00315	Zolmitriptan	5-hydroxytryptamine receptor 1B, P28222 5-hydroxytryptamine receptor 1D, P28221 5-hydroxytryptamine receptor 1F, P30939 5-hydroxytryptamine receptor 1A, P08908
DB00394	Beclomethasone	Glucocorticoid receptor, P04150
DB00588	Fluticasone Propionate	Glucocorticoid receptor, P04150 Progesterone receptor, P06401 Cytosolic phospholipase, A2 P47712 Mineralocorticoid receptor, P08235
DB00635	Prednisone	Glucocorticoid receptor, P04150 Corticosteroid 11-beta-dehydrogenase isozyme 1, P28845
DB00716	Nedocromil	Cysteinyl leukotriene receptor 1, Q9Y271 Cysteinyl leukotriene receptor 2, Q9NS75 Met-Leu-Phe receptor, P21462 Prostaglandin D2 receptor, Q13258 Heat shock protein HSP 90-alpha, P07900
DB00814	Meloxicam	Prostaglandin G/H synthase 2, P35354 Prostaglandin G/H synthase 1, P23219
DB00860	Prednisolone	Glucocorticoid receptor, P04150
DB00918	Almotriptan	5-hydroxytryptamine receptor 1D, P28221 5-hydroxytryptamine receptor 1B, P28222

Table 1 to be continued

DB00953	Rizatriptan	5-hydroxytryptamine receptor 1D, P28221 5-hydroxytryptamine receptor 1B, P28222 5-hydroxytryptamine receptor 1F, P30939
DB00998	Frovatriptan	5-hydroxytryptamine receptor 1D, P28221 5-hydroxytryptamine receptor 1B, P28222
DB01025	Amlexanox	Protein S100-A12, P80511 Protein S100-A13, Q99584 Interleukin-3, P08700 Fibroblast growth factor 1, P05230
DB01234	Dexamethasone	Glucocorticoid receptor, P04150 Nuclear receptor subfamily 0 group B member 1, P51843 Annexin A1, P04083 Nitric oxide synthase, inducible, P35228
DB00250	Dapsone	Inactive dihydropteroate synthase 2, P0C0X2 Dihydropteroate synthase 1, P0C0X1
DB01097	Leflunomide	Dihydroorotate dehydrogenase (quinone), mitochondrial, Q02127 Aryl hydrocarbon receptor, P35869 Protein-tyrosine kinase 2-beta, Q14289

After *in silico* analysis arepresentative sample of 15% of identified compounds was selected to validating their antileishmanial activity by in vitro assays (Table 4). Ten compounds (43.5%) were selected based on the possibility to target specific proteins in *Leishmania* spp, eight compounds (34.8%) were selected based on their antiprotozoal activity (including antileishmanial), four compounds (17.4%) were selected based on their properties as anti-inflammatory, wound healing or skin protector and one compound (4.3%) was selected because was able to target a specific protein in *Leishmani*a spp and also had antiprotozoal activity.

The cytotoxic effect of selected compounds was assessed in human macrophages (U-937), haPM (hamster's peritoneal macrophages) and human hepatic cells (HepG2). Results are summarized in Table 4. The authors observe fludrocortisone, bepridil, salicylhydroxamic acid, docosanol, pranlukast, marimastat, phenylbutazone, diclofenac, carbenoxolone, and nepafenac were cytotoxic to U-937 but not cytotoxic to haPM. Contrary, homatropine methylbromide and pamidronate were cytotoxic to haPM but non cytotoxic to U-937. Azelaic acid, propantheline, eucalyptol, bentoquatam, dapsone and pantoprazole, were not cytotoxic to U-937 neither to haPM. Adapalene, T198765, imatinib, alendronate, primaquine, amphotericin B and doxorribosin were cytotoxic to U-937 ad haMP. Adapalene, T198765, imatinib, diclofenac, dapsone, carbenoxolone, pantoprazole, pamidronate, primaquine and amphotericin B were cytotoxic to HepG2.

Seven of 23 (30.4%) compounds (Adapalene, T198765, bepridil, aalicylhydroxamic acid, phenylbutazone, bentoquatam, primaquine) showed high antileishmanial activity with EC_{50} < 20 µg/mL; other three compounds (Azelaic acid, propantheline, nepafenac) showed moderate antileishmanial activity with EC_{50} > 20 y < 50 µg/mL. The remaining compounds (Alendronate, carbenoxolone, fludrocortisone, docosanol, pranlukast, marimastat, imatinib, homatropine methylbromide, eucalyptol, diclofenac, dapsone, pantoprazole and pamidronate) were not active against intracellular amastigotes of *L. panamensis* with EC_{50} > 50 (Table 5). Bentoquantam, adapalene, primaquine, salycilhydroxamic acid, alendronate and carbenoxolene and nepafenac had TI also named IS (index of selectivity) higher than 1.0. The most selective compound was bentoquantam with a TI higher than 74 followed by Adapalene and Primaquine with TI $\geq$ 5.0 (Table 5).

Adapalene, bepridil, azelaic Acid, salicylhydroxamic acid, alendronate, phenylbutazone, propantheline bromure and bentoquatam were tested

in vivo because they showed activity against *L. panamensis* in vitro. Response to each treatment is summarized in Fig. 2.

At PTD30, 40% of cure was observed in hamsters of group bentoquantam 5% while only 20% of cure was observed in hamsters treated with adapalene 1%, alendronate (4% topical and oral formulations) and propanteline bromide 0.5%. Improvement of the lesion, with reduction between 52.4% and 84.3%, was obtained in 80% of hamsters when they were treated with azelaic acid 4% and propanteline bromide 0.5%.

Treatment with topical Alendronate 1% only produced improvement in 20% of hamsters. In turn, treatment with intralesional MA produce cure in 100% of animals in this group (Table 6). At PTD90, an increase from 20% to 60% in the cure was observed for adapalene 1%, alendronate (topic 4% and oral), bepridil 2.3% and propanteline bromide 0.5% and from 0% to 40% in hamsters treated with azelaic acid (Table 6). Treatment with topic salicylhydroxamic acid only produced improvement with reduction of lesion size ranking from 56% and 95%.Animals treated with adapalene, bentoquantam, bepridil, propantheline bromide, azelaic acid and salicylhydroxamic acid that did not cure had 20,420, 17,492, 5,050, 3,341, 4,374 and 9,478 parasites/mg, respectively. Differences were no statistically significant ($p > 0.05$).

Table 2 List of drugs with antiprotozoal activity detected *in silico* according to PASS structure.

Medicamento	Pa	Pi
Propantheline		
Anti-*Leishmania*	0.459	0.033
Antiprotozoal	0.280	0.099
Phenylbutazone		
Anti-*Leishmania*	0.439	0.048
Anti-*Toxoplasma*	0.340	0.183
Anti-Coccidia	0.237	0.067
Homatropine methylbromide		
Anti-*Leishmania*	0.417	0.072
Antiprotozoal	0.355	0.067
Eucalyptol		
Anti-*Leishmania*	0.413	0.076
Anti-*Plasmodium*	0.663	0.003
Anti-Coccidia	0.261	0.052
Pirenzepine		
Anti-Amoeba	0.302	0.074
Anti-*Trichomona*	0.245	0.094
Medrysone		
Anti-Amoeba	0.340	0.046
Anti-*Trichomona*	0.279	0.051
Anti-*Leishmania*	0.274	0.252
Cortisone acetate		
Anti-*Trichomona*	0.271	0.059
Anti-Amoeba	0.249	0.126
Fludrocortisone		
Anti-*Trichomona*	0.219	0.149

Table 2 to be continued

Nepafenac		
Anti-*Toxoplasma*	0.425	0.128
Anti-*Trypanosoma*	0.239	0.198
Anti-Coccidia	0.236	0.067
Anti-*Babesia*	0.110	0.107
Fluorometholone		
Anti-*Trichomona*	0.269	0.061
Anti-Amoeba	0.201	0.197
Diclofenac		
Anti-*Toxoplasma*	0.401	0.141
Anti-Coccidi	0.333	0.024
Anti-*Plasmodium*	0.198	0.168
Hydrocortamate		
Anti-*Trichomona*	0.265	0.065
Anti-Amoeba	0.221	0.161
Methylprednisolone		
Anti-*Trichomona*	0.272	0.058
Anti-Amoeba	0.269	0.104
Sulindac		
Anti-Amoeba	0.357	0.036
Thiamine		
Anti-*Trichomona*	0.300	0.036
Antiprotozoal	0.240	0.135
Anti-*Histomona*	0.053	0.030
Pamidronate		
Anti-*Trypanosoma*	0.696	0.004
Antiprotozoal	0.365	0.063
Zolmitriptan		
Anti-Amoeba	0.237	0.139
Prednisone		
Anti-*Trichomona*	0.229	0.126
Anti-Amoeba	0.206	0.187
Meloxicam		
Anti-Trichomona	0.306	0.032
Anti-Amoeba	0.211	0.179
Anti-*Histomona*	0.064	0.023
Prednisolone		
Anti-*Trichomona*	0.224	0.137
Leflunomide		
Anti-Coccidia	0.204	0.099
Anti-*Babesia*	0.116	0.096
Dapsone		
Anti-*Toxoplasma*	0.973	0.002
Anti-*Trypanosoma*	0.462	0.039
Antiprotozoal	0.445	0.037
Anti-*Plasmodium*	0.429	0.005

Table 3 List of drugs detected *in silico* with activity against putative target in *Leishmania*.

Code DB/CID	Drug	Target	Pa
Antibacterial			
DB01059/CID000004539	Norfloxacin	LinJ14.1250 enolase (429 aa)	0.598
		LinJ30.2220 hypothetical protein (180 aa)	0.500
		LinJ15.1220 mitochondrial DNA topoisomerase II (1236 aa)	0.416
DB01165/CID000004583	Ofloxaxin	LinJ14.1250 enolase (429 aa)	0.633
		LinJ30.2220 hypothetical protein (180 aa)	0.498
DB00713/CID000004607	Oxacillin	LinJ33.2040 DNA polymerase delta catalytic subunit (1032 aa)	0.476
		LinJ15.1410proliferative cell nuclear antigen (293 aa)	0.475
DB00607/CID000008982	Nafcillin	LinJ26.0120 adenine phosphoribosyltransferase (237 aa)	0.486
DB08798/CID000012894	Sulfamoxole	LinJ28.3060 glutamate dehydrogenase (452 aa)	0.499
Anti-inflammatory			
DB00152/CID000001130	Thiamine	LinJ30.11104-methyl-5(beta-hydroxyethyl)-thiazole monophosphate synthesis protein (196 aa)	0.810
		LinJ34.2770 putative pyruvate/indole-pyruvate carboxylase (583 aa)	0.789
		LinJ36.1010 dihydrolipoamide acetyltransferase precursor (463 aa)	0.599
		LinJ36.5900 selenophosphate synthetase (398 aa)	0.582
		LinJ27.0650 cysteine desulfurase (440 aa)	0.479
		LinJ36.3300 2-oxoglutarate dehydrogenase E1 component (1012 aa)	0.464
		LinJ18.1370 pyruvate dehydrogenase E1 component alpha subunit, putative (378 aa)	0.404
		LinJ27.0520 2-oxoglutarate dehydrogenase subunit (1006 aa)	0.402
DB00328/CID000003715	Indomethacin	LinJ17.0280 cystathionine beta-synthase (359 aa)	0.590
		LinJ12.0100 ornithine decarboxylase, putative (707 aa)	0.565
		LinJ14.1450 myo-inositol-1-phosphate synthase (417 aa)	0.470
		LinJ17.1520 myo-inositol-1(or 4)-monophosphatase 1, putative (287 aa)	0.405
DB00282/CID000004673	Pamidronate	LinJ35.1590 farnesyl pyrophosphate synthase (362 aa)	0.923
DB00687/CID000031378	Fludrocortisone	LmjF.01.0070 hypothetical protein, conserved	No reported
		LmjF.04.0570 hypothetical protein, conserved	No reported
DB00210/CID000060164	Adapalene	LinJ.27.2040 hypothetical protein, conserved	No reported
DB00580/CID000119607	Valdecoxib	LinJ06.0630 carbonic anhydrase family protein (306 aa)	0.604
Antiprotozoal			
DB00916/CID000004173	Metronidazole	LinJ04.0580 spermidine synthase, putative (300 aa)	0.447
DB00738/CID000004735	Pentamidine	LinJ12.0100 ornithine decarboxylase, putative (707 aa)	0.513
		LinJ34.0950 p-glycoprotein (1341 aa)	0.511

Table 3 to be continued

DB00205/CID000004993	Pyrimethamine	LinJ06.0890 dihydrofolate reductase-thymidylate synthase (395 aa)	0.968
		LinJ26.0040 glycine dehydrogenase, putative (973 aa)	0.709
		LinJ35.4840 glycine cleavage system H protein (107 aa)	0.704
		LinJ14.1410 serine hydroxymethyltransferase, putative; Interconversion of serine and glycine (465 aa)	0.475
		LinJ28.2470 serine hydroxymethyltransferase; Interconversion of serine and glycine (474 aa)	0.448
DB01117/CID000074989	Atovaquone	LinJ35.1590 farnesyl pyrophosphate synthase (362 aa)	0.447
Antiviral			
DB01004/CID000003454	Ganciclovir	LinJ21.0880 thymidine kinase (284 aa)	0.605
DB00442/CID000153941	Entecavir	LinJ12.0580 alanine aminotransferase (497 aa)	0.496
DB00632/CID000012620	Doconasol.	LmjF.09.0340 hypotheticalprotein, conserved	No reported
		LmjF.19.0450 hypotheticalprotein, conserved	No reported
		LmjF.32.2800 hypotheticalprotein, conserved	No reported
		LmjF.35.4120 hypotheticalprotein, conserved	No reported
		LinJ.03.0540 hypotheticalprotein	No reported
		LinJ.12.0662 surfaceantigenprotein 2, putative	No reported
		LinJ.17.1270 hypotheticalprotein, unknownfunction	No reported
		LinJ.24.0430 hypotheticalprotein, unknownfunction	No reported
		LinJ.27.2040 hypotheticalprotein, conserved	No reported
		LinJ.30.3540 chromosomal passenger protein, putative (CPC1)	No reported
		LinJ.32.3740 hypothetical protein, conserved	No reported
		LinJ.33.2780 hypothetical protein, conserved	No reported
		LbrM.07.0600 hypothetical protein, conserved	No reported
		LbrM.08.0550 hypothetical protein, conserved	No reported
		LbrM.16.1110 hypothetical protein, conserved	No reported
		LbrM.16.1740 hypothetical protein	No reported
		LbrM.19.1210 hypothetical protein, conserved	No reported
		LbrM.21.1400 hypothetical protein, conserved	No reported
		LbrM.24.0810 hypothetical protein, conserved	No reported
		LbrM.30.0230 hypothetical protein, conserved	No reported
Antitumoral, anti-neoplasic			
DB01128/CID000002375	Bicalutamide	LinJ29.0210 aminopeptidase (380 aa)	0.537

Table 3 to be continued

DB00548/CID000002266	Azelaic acid	LmjF.17.1100 3-oxo-5-alpha-steroid 4-dehydrogenase-like protein	No reported
		LmjF.21.1660 mitochondrial structure specific endonuclease I, putative	No reported
		LinJ.17.1200 3-oxo-5-alpha-steroid 4-dehydrogenase-like protein	No reported
		LinJ.21.2020 mitochondrial structure specific endonuclease I, putative	No reported
		LbrM.14.0890 mitochondrial DNA polymerase I protein C, putative	No reported
		LbrM.21.1950 mitochondrial SSE-1 (structure specific endonuclease I), putative	No reported
DB00619/CID000005291	Imatinib	LmjF.19.0450 hypotheticalprotein, conserved	No reported
		LinJ.17.1270 hypotheticalprotein, unknownfunction	No reported
		LbrM.21.1400 hypotheticalprotein, conserved	No reported
DB00786/000119031	Marimastat	LinJ.27.2040 hypotheticalprotein, conserved	No reported
		LbrM.16.1740 hypotheticalprotein	No reported
Antifungal			
DB01110/CID000004189	Miconazole	LinJ06.0670 lanosterol synthase (1007 aa)	0.496
DB03819/CID000066644	Salicylhydroxamic acid	LmjF.21.1567 hypotheticalprotein, conserved	No reported
		LmjF.34.0070 ascorbate peroxidase	No reported
		LinJ.21.1900 hypotheticalprotein, conserved	No reported
		LinJ.34.0070 ascorbate-dependent peroxidase, putative	No reported
		LbrM.20.0150 ascorbate-dependent peroxidase, putative	No reported
		LbrM.21.1780 hypotheticalprotein, conserved	No reported
DB04794/CID000000795	Imidazole	LinJ27.0220 acyl carrier protein; Carrier of the growing fatty acid chain in fatty acid biosynthesis (150 aa)	0.845
		LinJ26.0310 C-1-tetrahydrofolate synthase, cytoplasmic, putative (298 aa)	0.701
		LinJ28.2230 A/G-specific adenine glycosylase (501 aa)	0.691
		LinJ34.0070 ascorbate-dependent peroxidase (303 aa)	0.636
		LinJ32.2070 NAD+ synthase (293 aa)	0.616
		LinJ28.3160 glucose 6-phosphate N-acetyltransferase (148 aa)	0.578
		LinJ30.1620 pyridoxal kinase (302 aa)	0.534
		LinJ35.5000 RAD51/dmc1 protein (287 aa)	0.502
Anti-hypertensive and other health problems			
DB00661/CID000002520	Verapamil	LinJ34.0950 p-glycoprotein (1341 aa)	0.812
		LinJ26.2650 ATP-binding cassette transporter-like protein (1267 aa)	0.728
		LinJ06.0760 threonine dehydratase-like protein (338 aa)	0.436
		LinJ23.0290 multidrug resistance protein (1569 aa)	0.426
		LinJ23.0240 ATP-binding cassette transporter(1562 aa)	0.412
		LinJ23.0230 ATP-binding cassette transporter(1570 aa)	0.405

Table 3 to be continued

DB01244/CID000002351	Bepridil	LmjF.19.0450 hypothetical protein, conserved	No reported
		LmjF.35.1630 hypothetical protein, conserved	No reported
		LmjF.36.2430 caltractin, putative	No reported
		LinJ.36.2560 caltractin, putative	No reported
		LbrM.14.0520 hypothetical protein, conserved	No reported
		LbrM.35.2640 caltractin, putative	No reported
DB00641/CID000054454	Simvastatin	LinJ30.3600 3-hydroxy-3-methylglutaryl-CoA reductase (434 aa)	0.937
		LinJ31.3660 farnesyltransferase (414 aa)	0.502
DB00925/CID000004768	Phenoxybenzamine	LinJ36.4910 tyrosine aminotransferase (448 aa)	0.520
		LinJ36.2300 hypothetical protein (259 aa)	0.460
Anticonvulsant			
DB00909/CID000005734	Zonisamide	LinJ06.0630 carbonic anhydrase family protein (306 aa)	0.708
Antiespasmodico			
DB01411/CID000115100	Pranlukast	LmjF.32.2800 hypotheticalprotein, conserved	No reported
		LmjF.35.4120 hypotheticalprotein, conserved	No reported
		LmjF.35.4450 predicted zinc fingerprotein	No reported
		LinJ.03.0540 hypotheticalprotein	No reported
		LinJ.12.0662 surfaceantigenprotein 2, putative	No reported
		LinJ.17.1270 hypotheticalprotein, unknownfunction	No reported
		LinJ.24.0430 hypotheticalprotein, unknownfunction	No reported
		LinJ.27.2040 hypotheticalprotein, conserved	No reported
		LinJ.30.3540 chromosomal passenger protein, putative (CPC1)	No reported
		LinJ.33.2780 hypothetical protein, conserved	No reported
		LbrM.07.0600 hypothetical protein, conserved	No reported
		LbrM.08.0550 hypothetical protein, conserved	No reported
		LbrM.16.1740 hypothetical protein	No reported
		LbrM.19.1210 hypothetical protein, conserved	No reported
		LbrM.21.1400 hypothetical protein, conserved	No reported
		LbrM.24.0810 hypothetical protein, conserved	No reported
		LbrM.32.3740 hypothetical protein, conserved	No reported

Table 3 to be continued

Nutraceutical			
DB01373/CID000000271	Calcium	LinJ04.0010 calcium-translocating P-type ATPase (1022 aa)	0.905
		LinJ16.0550 aspartate carbamoyltransferase, putative (327 aa)	0.879
		LinJ17.0690 p-type ATPase (1134 aa)	0.830
		LinJ24.1450 transketolase (671 aa)	0.825
		LinJ33.2340 isocitrate dehydrogenase (425 aa)	0.809
		LinJ17.1520 myo-inositol-1(or 4)-monophosphatase 1, putative (287 aa)	0.808
		LinJ07.1110 cation-transporting ATPase, putative (1244 aa)	0.792
		LinJ14.1250 enolase (429 aa)	0.750
		LinJ31.3280 calreticulin (400 aa)	0.743
		LinJ09.0980 calmodulin (149 aa)	0.737
		LinJ09.0970 calmodulin, putative (149 aa)	0.737
		LinJ30.3860 PAS-domain containing phosphoglycerate kinase (527 aa)	0.718
		LinJ18.0510 aconitase, putative (896 aa)	0.716
		LinJ31.1020 NADH-ubiquinone oxidoreductase (201 aa)	0.712
		LinJ05.0990 NADH-ubiquinone oxidoreductase, mitochondrial, putative (481 aa)	0.712
		LinJ27.2100 glycosomal phosphoenolpyruvate carboxykinase (525 aa)	0.706
DB00630/CID000002088	Alendronate	LmjF.22.1360 farnesyl pyrophosphate synthase	No reported
		LinJ.22.1210 farnesyl pyrophosphate synthase	No reported
		LbrM.22.1240 farnesyl pyrophosphate synthase	No reported
Experimental			
DB02857/CID000000765	Guanosine	LinJ22.0110 guanosine monophosphate synthetase (656 aa)	0.840
		LinJ29.1050 guanine deaminase (454 aa)	0.794
		LinJ19.1480 inosine-5'-monophosphate dehydrogenase (514 aa)	0.777
		LinJ33.1120 guanylate kinase (203 aa)	0.742
		LinJ05.0840 methylthioadenosine phosphorylase (306 aa)	0.617
		LinJ32.3460 nucleoside diphosphate kinase b (151 aa)	0.606
		LinJ28.0930 ribonucleoside-diphosphate reductase large chain; Provides the precursors necessary for DNA synthesis (799 aa)	0.594
		LinJ25.2230 succinyl-CoA synthetase alpha subunit (299 aa)	0.577
DB02857/CID000000765	9-beta-D-arabinofuranosylguanine	LinJ29.1050 guanine deaminase (454 aa)	0.709
		LinJ05.0840 methylthioadenosine phosphorylase (306 aa)	0.708
		LinJ33.1120 guanylate kinase (203 aa)	0.706
		LinJ22.0110guanosine monophosphate synthetase (656 aa)	0.659

Table 3 to be continued

DB02857/CID000000765	9-beta-D-arabinofuranosylguanine	LinJ29.3130 inosine-adenosine-guanosine-nucleoside hydrolase (333 aa)	0.648
		LinJ28.2230 A/G-specific adenine glycosylase (501 aa)	0.608
		LinJ28.0930 ribonucleoside-diphosphate reductase large chain; Provides the precursors necessary for DNA syn (799 aa)	0.576
		LinJ34.3320 phosphomannomutase-like protein (593 aa)	0.548
		LinJ30.1250 adenosine kinase (345 aa)	0.478
		LinJ27.0330 ribokinase (329 aa)	0.462
		LinJ12.0130xanthine phosphoribosyltransferase (216 aa)	0.432
		LinJ10.1350 nucleoside phosphorylase-like protein (341 aa)	0.429
DB01819/CID000001005	Phosphoenolpyruvate	LinJ14.1250 enolase (429 aa)	0.998
		LinJ35.0090 pyruvate kinase (507 aa)	0.996
		LinJ35.0120 pyruvate kinase (454 aa)	0.996
		LinJ11.1000 pyruvate phosphate dikinase, putative (914 aa)	0.993
		LinJ27.1470 glycosomal phosphoenolpyruvate carboxykinase (525 aa)	0.986
		LinJ04.1180 fructose-1,6-bisphosphatase, cytosolic, putative (351 aa)	0.979
		LinJ29.2870 6-phospho-1-fructokinase (486 aa)	0.902
		LinJ27.2100 glycosomal phosphoenolpyruvate carboxykinase (525 aa)	0.867
		LinJ28.3010 cytosolic malate dehydrogenase (324 aa)	0.860
		LinJ35.3180 glycerol kinase, glycosomal (512 aa)	0.827
		LinJ10.0710 dihydroxyacetone kinase 1-like protein (589 aa)	0.824
		LinJ24.1450 transketolase (671 aa)	0.791
		LinJ18.1370 pyruvate dehydrogenase E1 component alpha subunit, putative (378 aa)	0.753
		LinJ34.2770 putative pyruvate/indole-pyruvate carboxylase (583 aa)	0.733
		LinJ28.1120 hypothetical protein (155 aa)	0.730
DB02577/CID000004973	Heme	LinJ17.1590 ferrochelatase-like protein (385 aa)	0.986
		LinJ23.1870 protoheme IX farnesyltransferase (433 aa)	0.976
		LinJ06.1320 coproporphyrinogen iii oxidase (301 aa)	0.922
		LinJ16.1380 cytochrome c; Electron carrier protein. (113 aa)	0.854
		LinJ06.1330 protoporphyrinogen oxidase-like protein (231 aa)	0.758
		LinJ32.3640 ATP-binding cassette transporter(704 aa)	0.732
		LinJ17.0280 cystathionine beta-synthase (359 aa)	0.713
		LinJ07.0430 cytochrome c1, heme protein, mitochondrial precursor, putative (258 aa)	0.674
		LinJ28.2810 cytochrome oxidase assembly protein-like protein (415 aa)	0.642
		LinJ18.0510 aconitase, putative (896 aa)	0.638

Table 3 to be continued

DB02577/CID000004973	Heme	LinJ33.1860 ATP-binding cassette transporter(640 aa)	0.513
		LinJ14.1450 myo-inositol-1-phosphate synthase (417 aa)	0.506
		LinJ35.0210 coatomer zeta subunit (184 aa)	0.492
		LinJ35.1280 NADH-dependent fumarate reductase (495 aa)	0.480
		LinJ09.1550 cytochrome b5-like (117 aa)	0.464
		LinJ34.0070 ascorbate-dependent peroxidase (303 aa)	0.450
		LinJ13.0990 NADH-cytochrome B5 reductase, putative (308 aa)	0.432
		LinJ33.2520 3-oxoacyl-acyl carrier protein synthase ii (459 aa)	0.412
DB04513/CID000005681	N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide	LinJ23.1520 lathosterol oxidase-like protein (302 aa)	0.564
DB02962/CID000005798	Benzimidazole	LinJ34.0070 ascorbate-dependent peroxidase (303 aa)	0.636
		LinJ31.1020 NADH-ubiquinone oxidoreductase (201 aa)	0.453
DB02522/CID000439811	Phosphonopyruvate	LinJ28.1650 phosphonopyruvate decarboxylase-like protein (415 aa)	0.700
DB03152/CID000445463	B-2-octylglucoside	LinJ07.0430 cytochrome c1, heme protein, mitochondrial precursor, putative (258 aa)	0.732
		LinJ34.3400 aquaporin 9 (230 aa)	0.459
DB03632/CID000449124	Argifin	LinJ13.1510 ras-family member, GTP-binding protein (365 aa)	0.606
		LinJ36.6380 small GTPase (364 aa)	0.602
DB01806/CID46936221	T198765	LmjF.17.1010, hydrolase, alpha/beta fold family-like protein	No reported
		LmjF.17.1040 hydrolase-like protein	No reported
		LmjF.17.1050 hydrolase-like protein	No reported
		LmjF.28.1570 hydrolase, alpha/beta fold family, putative	No reported
		LinJ.17.1110 hydrolase, alpha/beta fold family-like protein	No reported
		LinJ.17.1140 hydrolase-like protein	No reported
		LinJ.17.1150 hydrolase-like protein,esterase-like protein	No reported
		LinJ.28.1700 hydrolase, alpha/beta fold family, putative	No reported
		LbrM.17.1120 hydrolase, alpha/beta fold family-like protein	No reported
		LbrM.17.1150 hydrolase-like protein	No reported
		LbrM.17.1160 hydrolase-like protein,esterase-like protein	No reported
		LbrM.17.1170 hydrolase-like protein,esterase-like protein	No reported
		LbrM.28.1740 hydrolase, alpha/beta fold family, putative	No reported
DB04074/CID000000049	Alpha-ketoisovalerate	LinJ27.1280 branched-chain amino acid aminotransferase (401 aa)	0.991
		LinJ21.1100 2-oxoisovalerate dehydrogenase alpha subunit (479 aa)	0.976
		LinJ35.0150 2-oxoisovalerate dehydrogenase beta subunit,mitochondrial precursor (366 aa)	0.965
		LinJ34.2770 putative pyruvate/indole-pyruvate carboxylase (583 aa)	0.876

Table 3 to be continued

	LinJ32.3870 dihydrolipoamide dehydrogenase (476 aa)	0.734
	LinJ18.0510 aconitase, putative (896 aa)	0.716
	LinJ30.2440 alcohol dehydrogenase (399 aa)	0.706
	LinJ28.2630 acyl-coa dehydrogenase (629 aa)	0.698
	LinJ23.1830 hypothetical protein (245 aa)	0.697
	LinJ23.0400 NADP-dependent alcohol dehydrogenase (352 aa)	0.692
	LinJ33.0520 d-xylulose reductase (349 aa)	0.616
	LinJ33.1420 cysteine conjugate beta-lyase, aminotransferase-like protein (414 aa)	0.605
	LinJ25.1800 pyruvate dehydrogenase E1 beta subunit (350 aa)	0.572
	LinJ28.2530 2-oxoglutarate dehydrogenase, E2 component, dihydrolipoamide succinyltransferase (389 aa)	0.527
	LinJ18.1370 pyruvate dehydrogenase E1 component alpha subunit, putative (378 aa)	0.525
	LinJ36.1010 dihydrolipoamide acetyltransferase precursor (463 aa)	0.509
	LinJ06.0760 threonine dehydratase-like protein (338 aa)	0.500
	LinJ36.6970 oxidoreductase (340 aa)	0.451
	LinJ36.3300 2-oxoglutarate dehydrogenase E1 component (1012 aa)	0.439

Note: aa: amino acid; NAD^+: Nicotinamide adenine dinucleotide (oxidized form); NADH: Nicotinamide adenine dinucleotide (reduced form).

Table 4 Cytotoxicity in vitro of compounds with potential antileishmanial activity predicted *in silico*.

Compound	CL_{50} (μg/mL)		
	U-937	HepG2	MPHa
Adapalene	29.5 ± 3.6	1.4 ± 0.28	31.1 ± 4.2
T198765	4.5 ± 1.2	87.5 ± 6.1	7.7 ± 0.7
Fludrocortisone	27.0 ± 1.0	> 200.0	> 200.0
Bepridil	2.0 ± 0.4	> 200.0	> 200.0
Azelaic acid	> 200.0	> 200.0	> 200.0
Salicylhydroxamic acid	21.7 ± 3.9	> 200.0	> 200.0
Docosanol	92.5 ± 14.9	> 200.0	> 200.0
Pranlukast	144.1 ± 26.2	> 200.0	> 200.0
Marimastat	10.8 ± 0.6	> 200.0	> 200.0
Imatinib	23.5 ± 4.9	18.0 ± 0.2	31.7 ± 1.2
Alendronate	120.6 + 1.4	> 200.0	33.1 ± 6.3
Phenylbutazone	143.4 ± 24.2	> 200.0	> 200.0
Homatropine methylbromide	> 200.0	> 200.0	101.7 ± 2.2
Propantheline	> 200.0	> 200.0	> 200.0
Eucalyptol	> 200.0	> 200.0	> 200.0
Bentoquatam	> 200.0	> 200.0	> 200.0
Diclofenac	16.7 ± 0.1	47.7 ± 1.5	> 200.0
Dapsone	> 200.0	170.9 ± 18.9	> 200.0
Carbenoxolone	78.0 ± 9.7	103.1 ± 2.1	> 200.0
Pantoprazole	> 200.0	130.9 ± 18.3	> 200.0
Pamidronate	> 200.0	25.2 ± 7.2	17.8 ± 1.9
Nepafenac	52.9 ± 0.7	> 200.0	> 200.0
Primaquine	11.4 ± 2.4	21.7 ± 2.3	37.4 ± 0.8
Amphotericin B	40.9 ± 1.1	28.0 ± 2.4	53.8 ± 7.0
Doxoribosin	0.1 ± 0.01	0.7 ± 0.4	21.29 ± 0.4

Note: Data represent the mean ± SD.

Table 5 In vitro antileishmanial and selective activities.

Compound	CE_{50} (μg/mL)	TI
Adapalene	5.8 ± 0.2	5.1
T198765	10.9 ± 2.8	< 1.0
Fludrocortisone	> 27.0	< 1.0
Bepridil	0.8 ± 0.1	2.5
Azelaic acid	43.5 ± 9.1	< 2.0
Salicylhydroxamic acid	6.1 ± 1.3	1.6
Docosanol	> 92.5	< 1.0
Pranlukast	> 100.0	< 1.4
Marimastat	> 10.8	< 1.0
Imatinib	> 23.5	< 1.0
Alendronate	65.2 ± 2.8	1.8
Phenylbutazone	16.2 ± 1.0	< 2.0
Homatropine methylbromide	> 100.0	< 2.0
Propantheline	47.5 ± 7.7	< 2.0
Eucalyptol	> 100.0	< 2.0

Table 5 to be continued

Bentoquatam	2.7	> 74.1
Diclofenac	> 16.7	< 2.0
Dapsone	> 100.0	< 2.0
Carbenoxolone	65.7	1.2
Pantoprazole	> 100.0	< 2.0
Pamidronate	> 100.0	< 2.0
Nepafenac	46.0 ± 1.5	1.2
Primaquine	2.3 ± 0.4	5.0
Amphotericin B	0.07 ± 0.004	584.3

Data represent the mean ± SD.

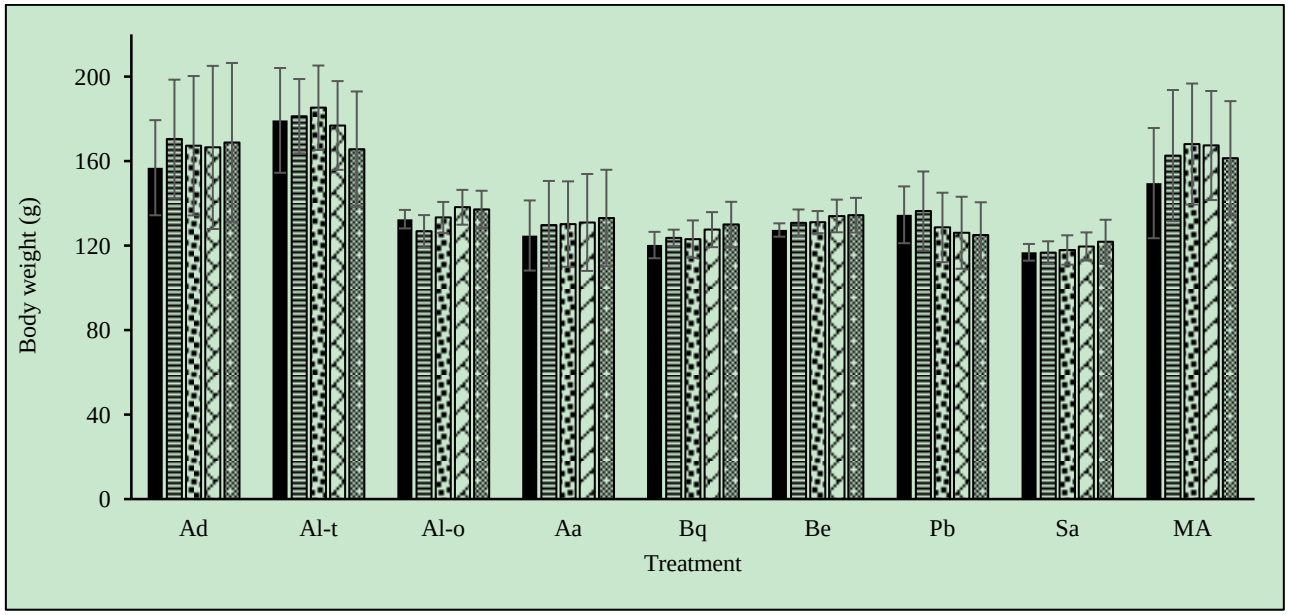

Fig. 2 Effect of treatment in body weight. Golden hamster (n = 6 in each group) were treated during 14 days Ad (adapalene), Al (alendronate), Aa (azelaic acid), Bq (bentoquantam), Be (bepridil), Pb (propanteline bromide), Sa (salicylhydroxamic acid) or MA. All treatments were administered topically, except alendronate that was administered also Al-o (alendronate) and MA that was administered intramuscular.

Table 6 Therapeutic efficacy according to clinical outcome of compounds with antileishmanial activity detected *in silico*.

Compound (route)[a]	Cure % (n)		Clinical improvement % (n)		Failure or relapse % (n)	
	PTD30[b]	PTD90	PTD30	PTD90	PTD30	PTD90
Adapalene (t)	20 (1)	60 (3)	0 (0)	0 (0)	80 (4)	40 (2)
Alendronate t (t)	20 (1)	60 (3)	0 (0)	0 (0)	80 (4)	40 (2)
Alendronate (o)	20 (1)	40 (2)	20 (1)	40 (2)	60 (3)	20 (1)
Azelaic acid (t)	0 (0)	0 (0)	80 (4)	80 (4)	20 (1)	20 (1)
Bentoquantam (t)	40 (2)	40 (2)	40 (2)	0 (0)	20 (1)	60 (3)
Bepridil (t)	20 (1)	60 (3)	60 (3)	0 (0)	20 (1)	40 (2)
Propanteline bromide (t)	20 (1)	60 (3)	40 (2)	40 (2)	40 (2)	0 (0)
Salicylhydroxamic acid (t)	0 (0)	0 (0)	80 (4)	60 (3)	20 (1)	40 (2)
Meglumine antimoniate (i.m)	100 (5)	80 (4)	0 (0)	0 (0)	0 (0)	20 (1)

[a] Route of administration; t: topic; o: oral; i.m: intramuscular; PTD: post-treatment day.

Table 7 Combined properties of compounds with potential antileishmanial activity detected *in silico*.

Compound (DB code)	Category[a] (Data minery)	Protozoal activity[b] Organism (Pa)	Target in *Leishmania*[c] (Protein code, Pa)	Antileishmanial activity in vitro (EC_{50})
Adapalene (DB00210)	Anti-inflammatory	No reported	LinJ.27.2040	High (5.8 μg/mL)
Phenylbutazone (DB00812)	Anti-inflammatory	*Leishmania* (0.439) *Toxoplasma* (0.340) Coccidia (0.237)	No reported	High (16.2 μg/mL)
Nepafenac (DB06802)	Anti-inflammatory	*Toxoplasma* (0.425) *Trypanosoma* (0.239) Coccidia (0.236) Babesia (0.110)	No reported	Moderate (46.0 μg/mL)
Eucalyptol (DB03852)	Anti-inflammatory	*Leishmania* (0.413) *Plasmodium* (0.663) Coccidia (0.261)	No reported	Low (> 100 μg/mL)
Dapsone (DB00250)	Anti-inflammatory	*Toxoplasma* (0.973) *Trypanosoma* (0.462) Protozoa (0.445) *Plasmodium* (0.429)	No reported	Low (> 100 μg/mL)
Diclofenac (DB00586)	Anti-inflammatory	*Toxoplasma* (0.401) Coccidia (0.333) *Plasmodium* (0.198)	No reported	Uknown (> 16.7 μg/mL, cytotoxic)
Propantheline (DB00782)	Skin lesions (anti-acne, anti-ulcerative/wound healing)	*Leishmania* (0.459)	No reported	Moderate (47.5 μg/mL)
Azelaic acid (DB00548)	Skin lesions (anti-acne, anti-ulcerative/wound healing)	No reported	LmjF.17.1100, LmjF.21.1660, LinJ.17.1200, LinJ.21.2020, LbrM.14.0890, LbrM.21.1950	Moderate (43.5 μg/mL)
Carbenoxolone (DB02329)	Skin lesions (anti-acne, anti-ulcerative/wound healing)	No reported	No reported	Low (65.7 μg/mL)
Homatropine Methylbromide (DB00725)	Skin lesions (anti-acne, anti-ulcerative/wound healing)	*Leishmania* (0.417) Protozoa (0.355)	No reported	None (> 100 μg/mL)
Pantoprazole (DB00213)	Skin lesions (anti-acne, anti-ulcerative/wound healing)	No reported	No reported	None (> 100μg/mL)
Bentoquatam (DB00516)	Skin protector	No reported	No reported	High (2.7 μg/mL)
Salicylhydroxamic Acid (DB03819)	Anti-mycotic	No reported	LmjF.21.1567, LinJ.21.1900 LbrM.21.1780, LmjF.34.0070, Li.34.0070, LbrM.20.0150	High (6.1 μg/mL)
Primaquine (DB01087)	Anti-protozoa	0.615 (Protozoa)	No reported	High (2.3 μg/mL)
Docosanol (DB00632)	Anti-viral	No reported	LmjF.09.0340, LmjF.19.0450, LmjF.32.2800, LmjF.35.4120, LinJ.03.0540, LinJ.12.0662, LinJ.17.1270, LinJ.24.0430, LinJ.27.2040, LinJ.30.3540, LinJ.32.3740, LinJ.33.2780, LbrM.07.0600, LbrM.08.0550, LbrM.16.1110, LbrM.16.1740, LbrM.19.1210, LbrM.21.1400, LbrM.24.0810, LbrM.30.0230	None (> 92.5 μg/mL)

Table 7 continued

Pamidronate (DB00282)	Anti-inflammatory, Anti-tumoral	*Trypanosoma* (0.696) Protozoa (0.356)	LinJ35.1590	None (> 100 μg/mL)
Imatinib (DB00619)	Anti-tumoral	No reported	LmjF.19.0450, LinJ.17.1270 y LbrM.21.1400	Unknown (> 23.5 μg/mL) cytotoxic (potencialmente citotóxico)
Marimastat (DB00786)	Anti-tumoral	No reported	LinJ.27.2040 y LbrM.16.1740	Unknown (> 10.8 μg/mL) cytotoxic (potencialmente citotóxico)
Bepridil (DB01244)	Anti-anginal	No reported	LmjF.36.2430, LinJ.36.2560 LbrM.35.26, LmjF.19.0450, LmjF.35.1630 LbrM.14.0520	High (0.8 μg/mL)
Pranlukast (DB01411)	Anti-spasmodic	No reported	LmjF.32.2800, LmjF.35.4120, LmjF.35.4450, LinJ.03.0540, LinJ.12.0662, LinJ.17.1270, LinJ.24.0430, LinJ.27.2040, LinJ.30.3540, LinJ.33.2780, LbrM.07.0600, LbrM.08.0550, LbrM.16.1740, LbrM.19.1210, LbrM.21.1400, LbrM.24.0810, LbrM.32.3740	None (> 100 μg/mL)
Alendronate (DB00630)	Bone Anti-resorptive	No reported	LmjF.22.1360, LinJ.22.1210 y LbrM.22.1240	Low (65.2 μg/mL)
T198765 (DB01806)	Experimental	No reported	LmjF.17.1010, LmjF.17.1040, LmjF.17.1050, LmjF.28.1570, LinJ.17.1110, LinJ.17.1140, LinJ.17.1150, LinJ.28.1700, LbrM.17.1120, LbrM.17.1150, LbrM.17.1160, LbrM.17.1170, LbrM.28.1740	High (10.9 μg/mL)

[a] DrugBank database; [b] PASS structure; [c] BLAST and STITCH.

On the other hand, no, significant changes in body weight of hamsters was observed in any group of treatment (Fig. 1). In addition, serum levels of ALT measured atTD8 were in the range of normal values while creatinine levels were slightly decreased in animals treated with alendronate and bentoquantam and BUN was increased in animals treated with adapalene, bepridil and meglumine antimoniate (Fig. 2). Histological alterations attributable to treatment were not also observed in animals treated with any of these compounds. In contrast, hamsters treated with MA induced moderate to severe cloudiness, vacuolar and fat degeneration, karyomegaly, bi-nucleation and pigmentation, in liver and, mild to moderate vacuolar and fat degeneration and bi-nucleation in kidney.

Overall, this work was focused on the identification of compounds that could be converted in candidates to potential drugs to treat CL using as strategy computational analysis of biological and biochemical properties of different drugs, specifically, antiparasite, anti-inflammatory and anti-ulcerative or wound healing activities but also the ability to target hypothetical or constitutive *Leishmania* proteins. In total 153 compounds were identified. Among these, four (2.6%) are used as skin protector, two compounds (1.3%) are anti-acne, 17 compounds (11.1%) are anti-ulcerative or wound healer, 64 (41.8%) are anti-inflammatory, 18 compounds (11.8%) show predicted antiprotozoal activity, four compounds (2.6%) have antileishmanial activity and 44 compounds (28.7%) that may inhibit specific conserved or hypothetical therapeutic target reported in *Leishmania* species.

The antileishmanial activity of 23 compounds (15% of identified by computational analysis) was tested in vitro. Twelve compounds (52.2%) showed high (EC_{50} < 20 μg/mL) or moderate (> 20 μg/mL to < 70 μg/mL)

activity. The most active compounds were anti-inflamatory (adapalene, phenyl brutazone and nepafenac), anti-acne, anti-ulcerative/wound healing (propanteline, azelaic acid and carboxolene), anti-protozoa (salicylhydroxamic acid and primaquine), skin protector (bentoquatam), anti-anginal (bepridil) bone-anti-resoption (alendronate) and one compounds that still is experimental (T198765). The therapeutic potential was validated in seven compounds that were evaluated in vivo. Adapalene, alendronate, azelaic acid, bentoquantam, bepridil, propanteline and salicylhydroxamic acid were able to induce cure or improvement of lesion of hamsters at the scheme tested here. Because these seven compounds showed antileishmanial activity both in vitro and in vivo, these results demonstrate that the in vitro and in vivo assays are well correlated. The antileishmanial activity of all these drugs could be explained by the fact that they are able to targetproteins presents in trypanosomatids including *Leishmania* or because their antiprotozoal activity based on chemical structure. Thus for example, Salicylhydroxamic acid inhibits the respiratory chain in *T. brucei brucei* and *T. vivax* [26].

Bepridil may block caltractin, a putative protein present in *L. braziliensis*, *L. major* and *L. infantum*. Recently was demonstrated the *in vitro* activity of bepridil against promastigotes of *L. major*, *L. chagasi* (syn. *L. infantum*), *L. braziliensis* and *L. amazonensis*, and intracellular amastigotes of *L. chagasi* [27]. Unfortunately, the compound was not effective in hamster with experimental VL, maybe due to a poor biodistribution of the formulation tested. Alendronatemay block human farnesyl pyrophosphate synthase, an enzyme of mevalonic acid pathway present in osteoclast and macrophages of bone tissues [28, 29]. This enzyme is also present in *L. donivani* and *L. major* and has been validated as a target for antileishmanial therapy using phosphonates as inhibitors of farnesyl pyrophosphate synthase [29, 30]. However, this is the first report of antileishmanial activity of alendronate. On the other hand, adapalene may target LinJ.27.2040, a hypothetic protein conserved in *L. infantum*. In addition, adapalene may modulate the immune response induced by *Leishmania* through interaction with nuclear receptors and affectation of gene transcription [31]. Notably, although adapalene was effective there are no reports of antileishmanial effect in the literature.

In turn, azelaic acid target various putative and-like proteins such as 3-oxo-5-alpha-steroid 4-dehydrogenase-like protein (LmjF.17.1100 and LinJ.17.1200), mitochondrial structure specific endonuclease I (SSE-1), putative (LmjF.21.1660, LinJ.21.2020 and LbrM.21.1950) and mitochondrial DNA polymerase I protein C, putative (LbrM.14.0890). Azelaic acid is widely used as a therapeutic agent in dermatology because its bactericidal activity [32-34]. However, the mechanism of this activity remains to be confirmed. Lastly, the antileishmanial activity of bentoquantam and propantheline bromide is not clear. Probably they may help to healing of damaged skin.

4. Conclusions

Bioinformatics tools not only can help to reduce time and cost of the drug discovery process but also may increase the chance that candidates identified *in silico* have a validated antileishmanial activity by combining different biological properties. In addition, focusing the search in molecules that have been approved as drugs, the possibility that the drug to be discarded during preclinical evaluation phase is also reduced. Furthermore, the drugs identified for a novel use can be modified or optimized to improve efficiency in a different pattern of illness, and in this particular case, the leishmaniasis, running as potential therapy forward for the treatment of leishmaniasis due to its low toxicity compared to the current treatment option.

The authors present here a strategy to identify second uses in commercially available drugs. As showed, the strategy has proven effective in finding

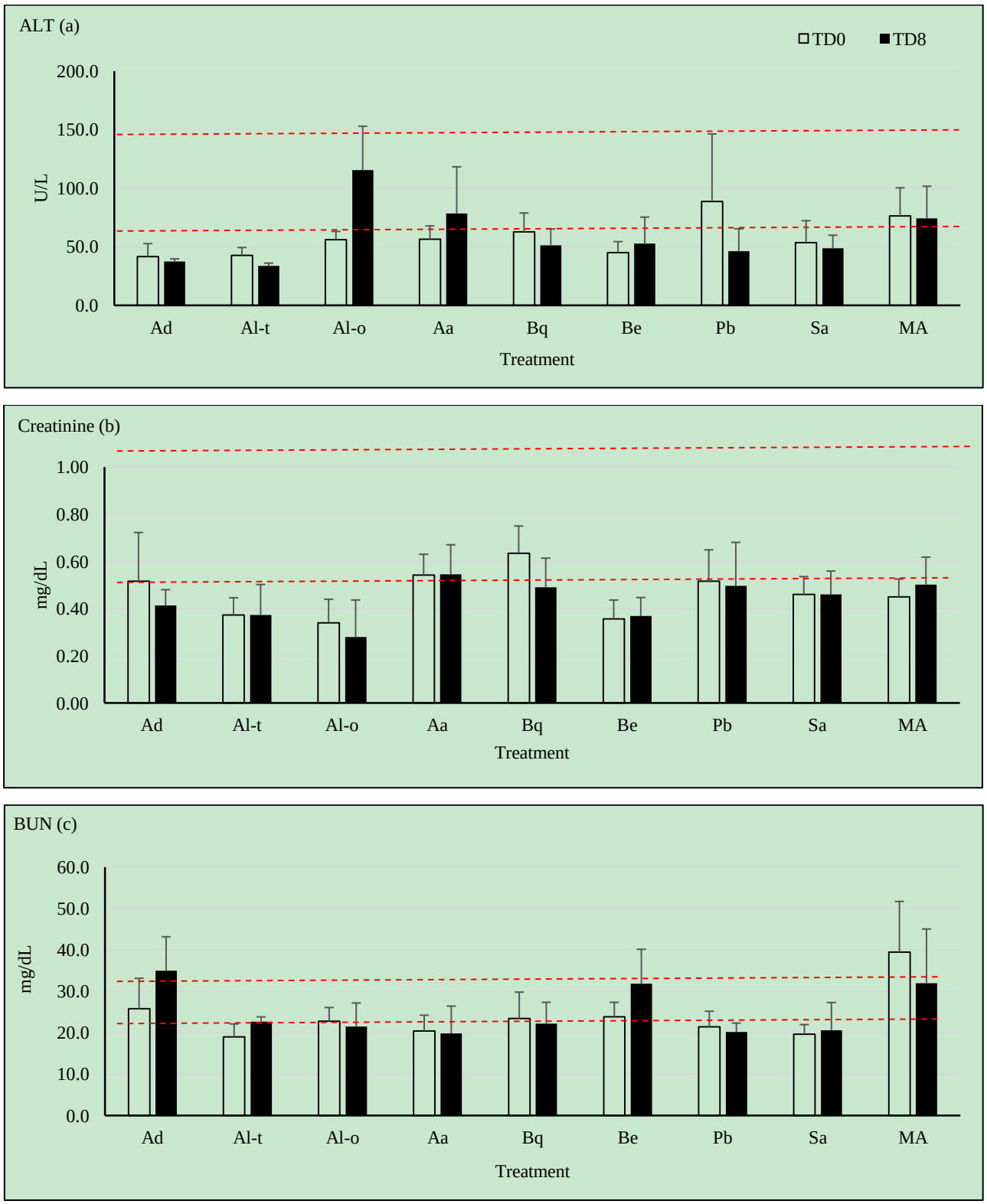

Fig. 3 Levels of ALT, creatinine and BUN in Serum in studied groups comparison of ALT (a), creatinine (b) and BUN (c) levels in serum of treatment groups receiving Ad, Al-t, Al-o, Aa, Bq, Be, Pb, Sa and MA. Data are shown as mean ± SD. No significant difference was seen between groups (P > 0.05).

potential drug candidates for leishmaniasis; however, this strategy can be approached for finding drug candidates for any human clinical condition. A caveat could be the lack of information about protein targets and mechanisms of action.

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