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Anti-Leishmanial Activity of a Library of Synthetic 4-Substituted 2-(1H-Pyrrolo [3, 2-c] Pyridin-2-Yl) Propan-2-Ols

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Abstract

Leishmaniasis is a devastating parasitic disease of medical and veterinary importance that is endemic across the tropics. It can be either disfiguring or lethal in its most severe forms and it lacks appropriate treatment. Several species of the protozoan parasite *Leishmania* can cause Leishmaniasis in human, which further complicates the development of new therapeutics because the different species are genotypically and phenotypically diverse. We have previously reported the anti-protozoal activity of 4-substituted 2-(1H-pyrrolo [3, 2-c] pyridin-2-yl) propan-2-ols against the related protozoan, *Trypanosoma cruzi*. Herein we report the biological activity of some of these compounds against intracellular amastigotes of three of the most clinically relevant Leishmania species: *L. amazonensis*, *L. braziliensis* and *L. infantum*. Four of the tested compound showed activity against the three Leishmania species, albeit with varied potency. The compounds were also cytotoxic to the human host cells at higher concentrations. A molecular modeling study suggested that these compounds are capable of binding to the ADP site and thus inhibit the leishmanial nucleoside diphosphate kinase (NDK). These results show that 4-substituted 2-(1H-pyrrolo [3, 2-c] pyridin-2-yl) propan-2-ols may potentially become chemical entities with broad-spectrum anti-leishmanial activity.

Keywords: Leishmania; Leishmaniasis; Kinetoplastids; Protozoan parasites; 2-(1H-pyrrolo [3, 2-c] pyridin-2-yl) propan-2-ol; 1H-pyrrolo [3, 2-c] pyridine; 5-azaindole; Nucleoside diphosphate kinase

Introduction

Leishmaniasis is a parasitic disease caused by kinetoplastid protozoan parasites of the genus *Leishmania*. About 12 million people are currently infected with Leishmaniasis [1] in 98 countries [2]. According to the World Health Organization, 2 million new cases [2] and between 20 and 30 thousand deaths occur each year [3]. In addition, approximately 200 million people in Asia, Africa, Central and South America and southern Europe live in areas where the disease is common [2,4]. The disease can present several clinical manifestations which range from skin lesions (Cutaneous Leishmaniasis – CL) and mucous ulcers (Mucocutaneous Leishmaniasis – MCL) to systemic visceral organ damage (Visceral Leishmaniasis – VL) [5,6]. Cutaneous Leishmaniasis is the most common form, which causes open sores to develop at the sites of the sand-fly bites. These open sores heal in 6 to 18 months, leaving behind severe scars [2,5]. Diffuse cutaneous Leishmaniasis produces lepromatous type lesions disseminated across the skin and can be more difficult to heal [2]. Mucocutaneous Leishmaniasis is characterized by damage to the mucosal membranes of the face and a profound inflammatory response, which can lead to the erosion of the nostrils and the mouth in particular [2,5]. Visceral Leishmaniasis, also known as kala-azar ('black fever') is the most severe form and is potentially fatal if untreated. Its manifestation includes damage to the spleen (splenomegaly) and liver (hepatomegaly) [5]. Infections in humans are caused by more than 20 species of *Leishmania*. Visceral disease is usually caused by *Leishmania donovani* or *L. infantum*, but occasionally these species may cause other forms of the disease, whereas cutaneous forms of Leishmaniasis are caused by more than 15 species of *Leishmania*, including *L. amazonensis*, *L. braziliensis* and *L. major* [2].

Because there are no vaccines for Leishmaniasis, the main strategy to control the disease is to treat infected individuals. However, the current arsenal of anti-leishmanials, which includes pentavalent antimonials, amphotericin B, paramomycin and miltefosine, is limited and has several disadvantages such as toxicity, variable efficacy regarding different species and geographic regions, requirements for parenteral administration, lengthy treatment regimens and the emergence of drug resistance [5]. Therefore, the identification of new and active compounds is urgent in order to develop new, safe and efficacious candidates for anti-leishmanial chemotherapy. In this context, new and established pharmacophores, based on synthetic and natural product chemistry, are being identified through improved screening technologies [2]. We recently reported the synthesis and evaluation of the biological activity of a library of 16 1H-pyrrolo [3, 2-c] pyridines (5-azaindoles), specifically 4-substituted 2-(1H-pyrrolo [3, 2-c] pyridin-2-yl) propan-2-ols (of which 11, 1–11, are shown in Figure 1 against *Trypanosoma cruzi*, the kinetoplastid protozoan parasite that causes Chagas disease. Three of the tested compounds presented relatively high trypanocidal activity; however, host cell toxicity was observed concomitantly [7]. In this work, we tested compounds 1-11 against different *Leishmania* species, because the kinetoplastid protozoan parasites that cause Leishmaniasis (*Leishmania ssp.*), human African trypanosomiasis (HAT) (*Trypanosoma brucei ssp.*) and Chagas disease (*Trypanosoma cruzi*) are taxonomically related and have similar structural and biochemical features [2]. The 4-substituted 2-(1H-pyrrolo [3, 2-c] pyridin-2-yl) propan-2-ols 1-11 displayed anti-leishmanial activity against *Leishmania infantum*, *L. amazonensis* and *L. braziliensis*, the results of which we present and discuss in this

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report. To evaluate the possible inhibitory activity against Leishmania nucleoside diphosphate kinase (NDK), a molecular docking study was performed on the nucleoside diphosphate kinase (NDK) from *L. braziliensis* (LbNDK). NDKs have been shown to be promising current targets for the design and discovery of anti-leishmanial drugs [8,9].

Materials and Methods

Cell culture

THP-1 cells (from the Rio de Janeiro Cell Bank, Xerem – RJ, Brazil) were cultured in RPMI 1640 media, supplemented with 20% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin, at 37°C in a 5% CO_2, humidified incubator. Cells were seeded at 2×10^5/mL every 3 to 4 days. *L. infantum* (MHOM/BR/72/BH046) were kindly provided by Prof Ricardo Fujiawara – UFMG, Brazil, while *L. amazonensis* (MHOM/BR/1977/LTB0016) and *L. braziliensis* (MHOM/BR/75/M2903) were obtained through the *Leishmania* repository of Fundação Oswaldo Cruz, Rio de Janeiro – RJ, Brazil. Promastigotes were cultured in M199 media supplemented with 40 mM Hepes, 0.1 mM adenine, 0.0001% biotin and 0.46 mM $NaHCO_3$, 10% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin. Parasites were passaged at 1×10^6/mL every 3 to 4 days in T175-flasks and maintained at 26°C under constant agitation (30 rpm).

Compounds

The synthesis of the library of the 16 4-substituted 2-(1*H*-pyrrolo [3, 2-*c*] pyridin-2-yl) propan-2-ols (of which 11, 1–11, are shown in Figure 1 is detailed in Balfour et al. [7].

High content screening assay

THP-1 cells were plated onto 384-well plates in 25 µL of RMPI complete media (2.8×10^5/mL) containing 50 ng/mL PMA and incubated at 37°C/5% CO_2 for 48 h. On infection day, 6-day-old promastigotes of *Leishmania infantum* (MHOM/BR/1972/BH46), *Leishmania amazonensis* (MHOM/BR/1977/LTB0016) or *Leishmania braziliensis* (MHOM/BR/75/M2903) were added for infection (1.4×10^7/mL) in 25 µL of media and the assay plates were placed at 37°C (34°C for cutaneous species) and 5% CO_2, humidified incubator. After 24 hours, negative controls (0.5% DMSO), positive controls (10 µM amphotericin B) or test compound were added into the plate. Prior to the addition of compound, samples and reference drug were serially diluted (dilution factor=2), starting from 100 µM. The plates were incubated for 96 h for *L. amazonensis* and *L. braziliensis* and 72 h for *L. infantum* and then were fixed with 4% paraformaldehyde and stained with 5 µM Draq5. The Operetta high-content automated imaging system (Perkin Elmer) was used to acquire images and the Harmony software (Perkin Elmer) was used for image analysis, with the following output parameters: THP-1 cell number, infection ratio and number of parasites per infected cell.

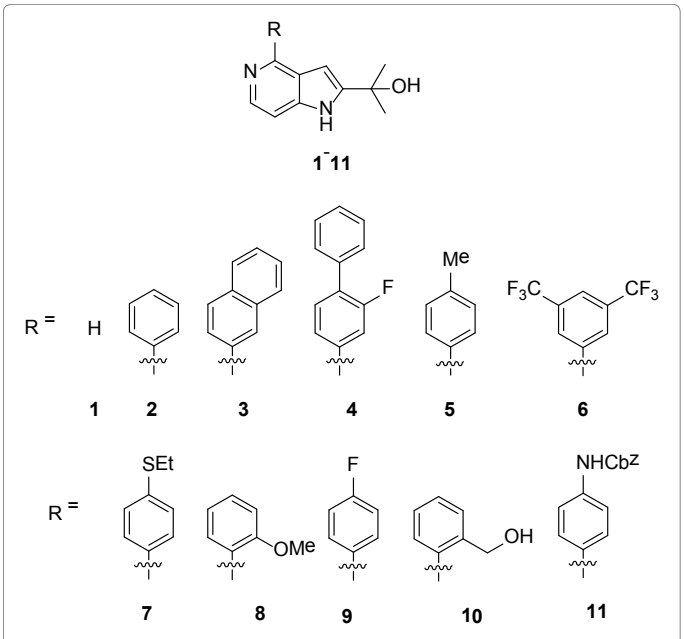

Figure 1: The library of 4-substituted 2-(1H-pyrrolo[3,2-c]pyridin-2-yl)propan-2-ols 1-11.

Data analysis

The infection ratio (IR) was defined as the ratio between (i) the total number of infected cells in all images from the well and (ii) the total number of cells in all images from the same well. IR was normalized to negative (infected cells, DMSO-mock treated) and positive (non-infected cells) controls to determine the normalized activity: NA=[1 – (Av. IRT – Av. IRP)/(Av. IRN – Av. IRP)] × 100. The cell ratio was defined as the ratio between the total number of cells in the test compound well to the average total number of cells from the negative control wells (infected cells, DMSO-mock treated). The cell ratio is an estimation of compound activity against the THP-1 host cell and is measured to estimate compound selectivity towards the *Leishmania* parasite. EC_{50} (concentration of compound that reduces infection by 50%), CC_{50} (concentration of compound that reduces the number of THP-1 cells by 50% in relation to infected, non-treated controls) and the selectivity index (SI, estimated as a ratio between CC_{50} and EC_{50}, or a ratio between the highest compound concentration tested and the EC_{50} whenever CC_{50} could not be calculated) were determined as described [7].

Molecular modeling

The tridimensional geometries of 3, 4, 7 and 11 were drawn in the ChemSketch platform [10] and then optimized to adjust to the MMFF94 force field [11] using the Avogadro program [12]. The nucleoside diphosphate kinase of *L. braziliensis* (PDB ID: 4KPC) [13] was chosen as the target structure. Prior to docking, its atomic coordinates were aligned with those of the *L. major* homologue in complex with ADP (PDB ID: 3NGU), [14] by employing the PyMol program [15]. Automated docking was performed with the AutoDock program, [16] keeping the protein fixed and allowing freely rotatable bonds for the ligands. The grid box was centered at the center-of-mass of ADP (three-dimensional coordinates retrieved from PDB ID: 3NGU) encompassing a volume of 1488 Å3 (X=28, Y=36 and Z=28 points). Discretization was set at 0.375 Å. The Lamarckian Genetic Algorithm (LGA) implemented in the Autodock program was applied using default parameters. The selection of docking ligand binding poses was based on the ranking of the ten ligand conformational clusters and their respective binding energy values. The visual analyses of ligand-protein complexes were made by the PyMOL program [15]. Two-dimensional diagrams were obtained with the Pose View program [17].

Results

Biological activity evaluation

A high content screening (HCS) assay was used for biological models of distinct *Leishmania* species causing cutaneous (*L.*

amazonensis), mucosal (*L. braziliensis*) and visceral *leishmaniasis* (*L. infantum*) in order to assess the compounds activity against intracellular amastigotes. All compounds were tested in dose-response. Among the set of compounds tested, 4 and 11 presented the highest activity at 50 μM, leading to an infection inhibition higher than 75%, for all *Leishmania* species (Figure 2).

Interestingly, 3 and 7 showed a species-dependent activity profile: while 3 was active specifically against *L. infantum* (approximately 100% of antiparasitic activity), 7 presented an activity which varied from 55 to 85%. Moreover, compounds were not cytotoxic at tested concentrations except for 11 which caused a cell ratio lower than 0.5, in both *L. amazonensis* and *L. braziliensis* models (data not shown). Regarding the antiparasitic activity (EC50) and the host cell cytotoxicity (CC50), compounds with high efficacy (high maximum activity) and relatively moderate potency (low EC50) against all three *Leishmania* species were identified; however, in general, the compounds presented a species-dependent activity. The order of sensitivity was: L. infantum>*L. amazonensis*>*L. braziliensis* (Table 1, Figure S1 Suppl). Among the tested compounds, compounds 3, 4, 7 and 11 exhibited high efficacies against the full species panel, with means of maximum activity of 95.4% ± 5.23, for *L. infantum*, 87.0% ± 11.8, for *L. amazonensis* and 61.4% ± 11.9, for *L. braziliensis*. On the other hand, compound 5 presented significant inhibitory activity only against L. infantum (95.5%) and moderate activity against *L. amazonensis* (61.9%) and compounds 6 and 9 displayed a discrete efficacy (20.9% and 48.0%) against *L. infantum* specifically. Compounds 1, 2, 8 and 10 were not active against any *Leishmania* species tested in this work. Only compounds 4 and 11 were also potent: while the former presented a consistent and moderate potency against all *Leishmania* models, with EC50 values in a range of 18.6 – 21.8 μM, the latter showed a species-dependent activity, with EC50 values of 6.8, 13.1 and 22.0 μM, against *L. infantum*, *L. amazonensis* and *L. braziliensis*, respectively. The compounds were not cytotoxic at the tested concentrations (CC50>100 μM), except for 4 and 11, where higher concentrations lead to host cell toxicity as shown by a decrease in cell number. Compound 4 presented a comparable cytotoxicity among the *Leishmania* models, generating a selectivity index of approximately 3, whereas compound 11 showed a higher toxicity in both *L. amazonensis* (CC50 30.8 μM/SI 1.4) and *L. braziliensis* (CC50 33.4/SI 2.4) models, compared to *L. infantum* (CC50 78.9 μM/SI 11.6). The dose response curves are shown in the supplementary material.

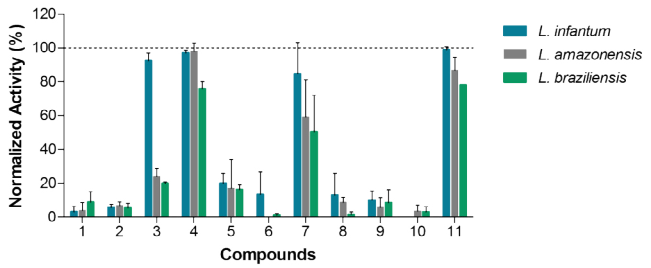

Figure 2: Profile of the tested compounds at 50 μM against distinct species of *Leishmania* intracellular amastigotes after 96 h of compound exposure (72 h for *L. infantum*) regarding antileishmanial activity (left) and host cell toxicity (right). Data are means and error bars represent standard deviations of two replicates. Bars colours indicate distinct species as shown in the legend.

Molecular docking

Nucleoside diphosphate kinases (NDKs) catalyze the transfer of a γ-phosphoryl group from a nucleoside triphosphate (NTP) donor to a nucleoside diphosphate (NDP) acceptor in a reversible mechanism. Therefore, NDKs are classified as ubiquitous enzymes that participate in different metabolic pathways, such as the regulation of gene expression in mammalian cells, bacterial pathogenesis and parasite housekeeping. In addition, NDKs are recognized to show highly conserved overall structure among their homologues, especially in relation to the active site [13,14]. These particular characteristics have made NDKs promising current targets for the design and discovery of antiparasitic drugs, including anti-leishmanial agents [8,9]. Taking these biological characteristics into consideration, the molecular modeling study was started using the atomic coordinates of the NDK of L. major in complex with ADP (PDB ID: 3NGU) to align the homologue *L. braziliensis* (PDB ID: 4KPC). The latter was chosen as the target structure because it was one of the *Leishmania* species employed in the biological testing. In addition, this study aimed to evaluate the possible mode of binding of 3, 4, 7 and 11 into the ADP site of the parasite enzyme. This hypothesis can be supported by visualization of the docking ligand binding poses of 3, 4, 7 and 11 (Figure 3). For compound 11, a binding mode different from that of all other compounds was predicted. Its lowest binding energy pose has the 4-phenyl-2-(1H-pyrrolo [3, 2-c] pyridin-2-yl) propan-2-ol moiety superimposed on the adenine and ribose rings of the crystallographic ADP molecule. This pose represents the first conformational cluster (Cluster 1). Conversely, in Cluster 2, the lowest

Compound	*L. infantum*				*L. amazonensis*				*L. braziliensis*			
	EC_{50}	CC_{50}	S.I.	M. A. (%)	EC_{50}	CC_{50}	S.I.	M. A. (%)	EC_{50}	CC_{50}	S.I.	M. A. (%)
Ampho B	2.3	>25	>10.9	101.9	1.8	>25	13.7	102.1	1	>25	>24.8	95.5
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-
3	29.8	>100	>3.4	94.1	62.4	>100	>1.6	89.9	81.9	98.8	1.2	58
4	18.6	53.9	2.9	96.1	21.7	62.9	2.9	100	21.8	67.6	3.1	78.7
5	71.4	>100	>1.4	95.5	85.1	>100	>1.2	61.9	91.3	>100	>1.1	48.8
6	-	-	-	20.9	-	-	-	-	-	-	-	-
7	35.6	>100	>2.8	89.5	46.4	>100	>2.2	71.5	37.7	>100	>2.7	52
8	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	48	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-
11	6.8	78.9	11.6	102.1	22	30.8	1.4	86.6	13.9	33.4	2.4	56.7

EC50 and CC50 values are expressed as averages (μM: Indicates that value was not determined; Max. Act: Maximum activity observed in the dose-response curve. S.I: Selectivity Index; n = 2 independent experiments.

Table 1: Biological activity of new compounds and reference drug against *Leishmania* intracellular parasites.

binding energy poses of 3, 4 and 7 have the 2-(1H-pyrrolo [3, 2-c] pyridin-2-yl) propan-2-ol moiety superimposing the phosphate groups of ADP. Remarkably, docking solutions corresponding to both clusters were obtained for all compounds, except for 4. The latter only yielded poses lying within Cluster 2, which is, in fact, the most populated one for all the compounds analyzed here (Table 2). In this table, it is possible to observe a narrow range between the binding energy values, in particular, their mean values, particularly for 3 and 7, which may explain the occurrence of these two principal conformational clusters.

Interestingly, 11 showed the lowest binding energy and occupied the ADP site in a similar mode to that which has been observed for recently identified Leishmanial NDK inhibitors [8,9]. The presence of similar structural characteristics between these inhibitors and 11 is noticeable. All these compounds have a heteroaromatic moiety (a hydrophobic region, the 2-(1H-pyrrolo [3, 2-c] pyridin-2-yl) propan-2-ol moiety of 11) attached to a side chain containing polar groups (a polar region), represented by the amino-carboxy benzyl group in 11. In addition, 11 possess the highest number of rotatable bonds, with higher number of degrees of freedom. These intrinsic characteristics allow 11 to show greater structural similarity with ADP, making its docking closer to that of the natural substrate.[14] Compounds 3, 4 and 7 have predominantly two hydrophobic regions, the 2-(1H-pyrrolo [3, 2-c] pyridin-2-yl) propan-2-ol moiety attached to the 4-(β-naphtyl) (3), 4-phenyl-(3'-fluoro-4'-phenyl) (4) and 4-phenyl-(4'-thioethyl) groups (7). Furthermore, due to the chemical nature of these 4'-substituent groups in 3 and 4, a major steric hindrance between the two hydrophobic regions is observed. In addition, the obstructive ortho effect promoted by the fluorine group in 4 may contribute to its preferential conformation in an inverse mode of ADP. These facts are readily described by two-dimensional diagrams [18] of 4 and 11 (Figure 4). For the sake of comparison, the corresponding diagram of ADP in the binding site of 3NGU was also included [14]. As can be seen in these diagrams, 11 interacts with the same hydrophobic (His50, Tyr51, Leu63, Val111, Gly112) and polar (Lys11, Asn114) residues that ADP interacts with, while it was the 3'-fluoro-4'-phenyl group in 4 that interacted with the same hydrophobic residues. These notes about the structural requirements may explain the weak or absent anti-leishmanial activity of 1, 2, 5, 8 and 10 observed in the biological testing. In these compounds the 4-substituent group is hydrogen (1), or a phenyl group without substituents (2), or substituted by a methyl group (5), or with polar groups in the ortho position (8 and 10). Therefore, these comparatively lower molecular structures would dock in the ADP binding site, with the 2-(1H-pyrrolo [3, 2-c] pyridin-2-yl) propan-2-ol moiety in particular, but probably with a significantly reduced number of polar and hydrophobic interactions, resulting in lower energy binding values.

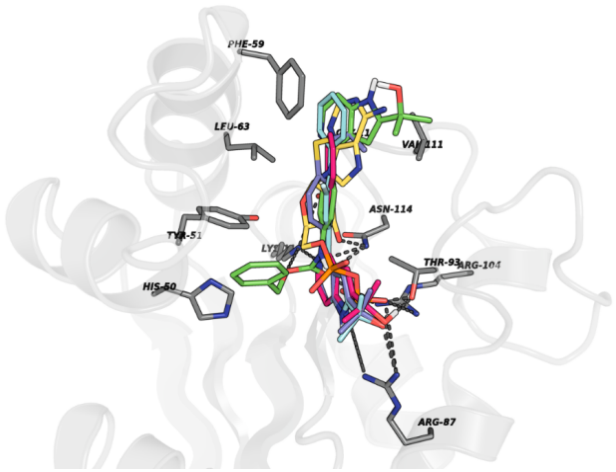

Figure 3: Superposition of the 4-substituted 2-(1H-pyrrolo [3, 2-c] pyridin-2-yl)-propan-2-ol compounds (3, 4, 7, and 11) to the co-crystallized ADP (retrieved from PDB ID: 3NGU) in the binding site of LbNDK. Carbon atoms of 3, 4, 7, and 11 are shown in magenta, light blue, cyan and green, respectively. Carbon atoms of ADP are shown in yellow. Gray dashed lines indicate H-bonding interactions. Only the polar hydrogen atoms of the ligand compounds were presented.

Discussion

In this work, a high content assay was used to assess the biological activity of a small library of 4-substituted 2-(1H-pyrrolo [3, 2-c]

Compound	Conformational cluster	Lowest binding energy (kcal/mol)	Mean binding Energy (kcal/mol)	Conformations/ Cluster
11	1	-6.99	-6.6	3
	2	-5.76	-5.29	6
3	1	-5.96	-5.96	1
	2	-6.17	-5.98	8
4	2	-6.67	-6.67	10
7	1	-5.09	-4.92	2
	2	-5.65	-5.4	8

Table 2: Conformational clusters, lowest and mean binding energy values, and number of conformations per cluster relative to the docking of 3, 4, 7, and 11 into the ADP site of *Lb*NDK.

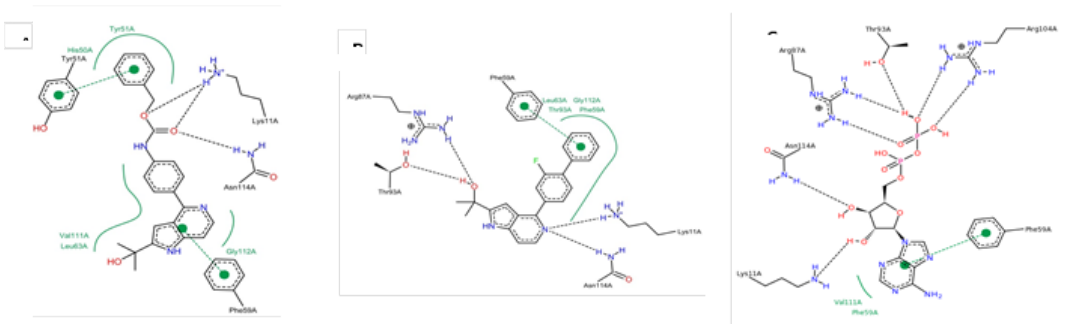

Figure 4: Two-dimensional diagrams of the molecular interactions between compounds 11 (A) and 4 (B) in the ADP binding site of the LbNDK. The ADP interactions reported in the 3NGU are presented for comparison (C). Black dashed lines indicate hydrogen bonds. Green solid lines show hydrophobic interactions, while green dashed lines show π-π interactions.

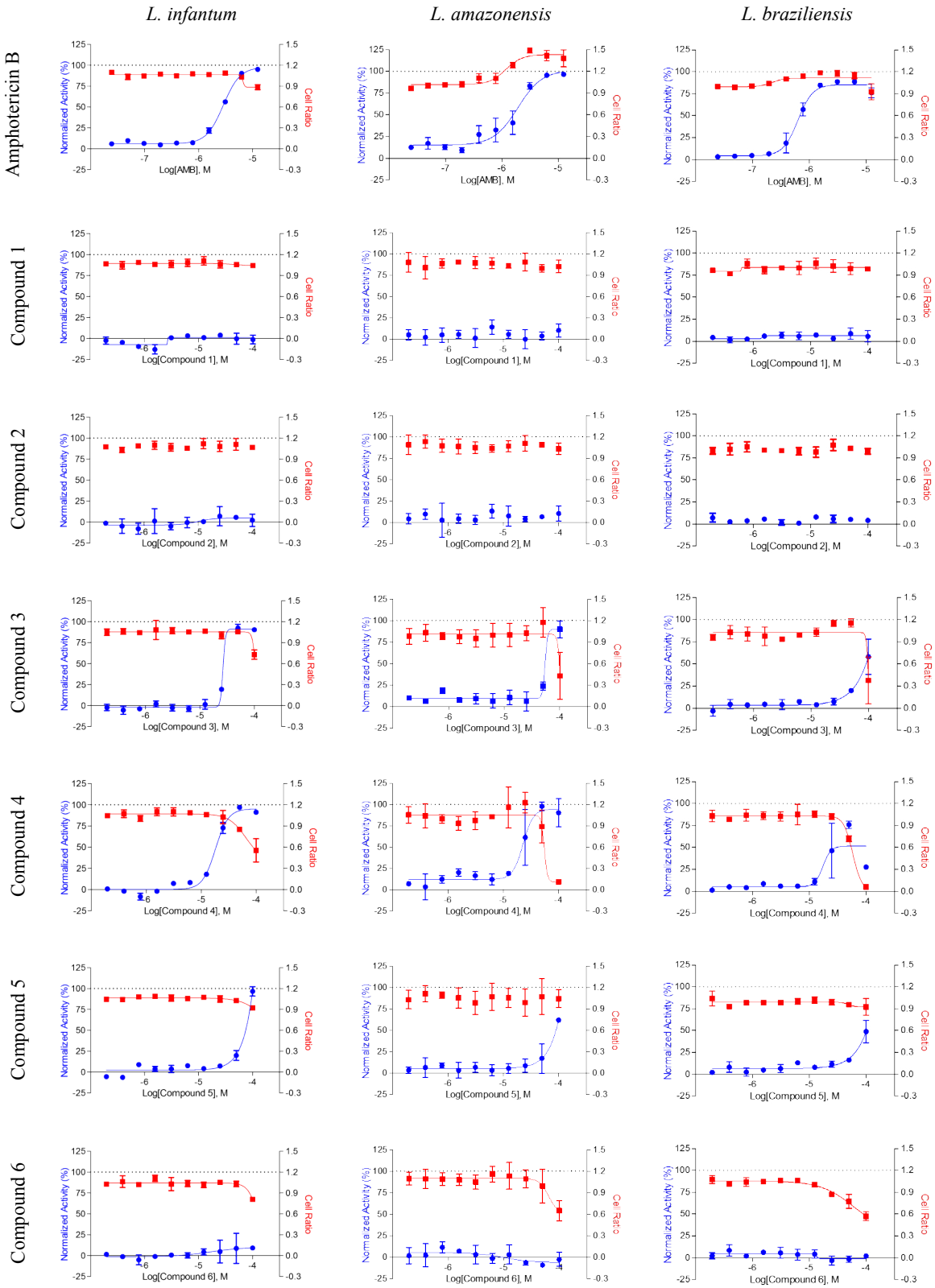

Figure S1: Dose-response curves: Curves were generated by a nonlinear regression equation (Graphpad prism). Graphs represent the normalized activity (blue color) and cell ratio (red color). Images represent data from two independent experiments.

pyridin-2-yl) propan-2-ols against intracellular amastigotes of three *Leishmania* species. The use of the HCS approach simultaneously provided information on the anti-leishmanial activity of the compounds against the disease-relevant life cycle stage of the parasite and toxicity against host cells. Additionally, compared to the frequently performed methodologies of manual counting and genetically modified parasites, image-based assays are considered more sensitive, robust and reliable [19-22].

The compounds tested in this work, particularly compounds 3, 4, 7 and 11, displayed reasonable activity against the full species panel, being generally more active against *L. infantum*, followed by *L. amazonensis* and then *L. braziliensis*. These compounds presented considerable efficacy against *Leishmania* parasites, with values of maximum activity higher than 52%, but only compounds 4 and 11 were also potent. However, the potency observed for these two compounds demonstrated a distinct profile: while compound 4 was comparably active against the full species panel (EC50 ~ 20 μM), possibly suggesting a common mechanism of action/target among the species, compound 11 presented variable potency against different *Leishmania* species. Previous studies have already reported evidence of intrinsic variation in *Leishmania* susceptibility among the different species of *Leishmania*, even for reference compounds. Escobar and colleagues, for example, have demonstrated that miltefosine potency varied from 2.6 μM for *L. aethiopica* to 37.2 μM for *L. major* [23]. The same divergent activity profile was also demonstrated for amphotericin B [24] and antimonial compounds [25-27]. Although not clearly elucidated, the differences in *Leishmania* sensitivities to reference and experimental drugs has been associated with several biochemical and molecular variations among the species, including the levels of protein expression and the biochemical composition of the plasma membrane [23,28]. Additionally, distinct *Leishmania* species and strains share significant differences in their genotype [29-32] gene expression regulation [33,34] infectivity, [35-38] and infection profile [23,39] which, indeed, could have an impact on drug activity.

Azaindoles, the chemical class used herein, have been frequently explored in drug discovery, since they have broad-spectrum biological activity and clinical applications, for example as benzodiazepine receptor ligands, [40] dopamine D4 receptor ligands, [41] and antineoplasic agents [42,43]. In the infectious disease field, studies have reported the inhibitory potential of azaindoles against the models of *Giardia duodenalis* [44] and *Plasmodium falciparum* [45] In a report by Dodd et al., a hybrid compound containing a thiosemicarbazone linked to an azaindole nucleus showed no anti-leishmanial activity at the up concentration tested [46]. On the other hand, considering the chemical structure of the general scaffold, there have been several reports of nitrogen containing heterocyclic compounds in general that display anti-leishmanial activity [47-52]. Moreover, compounds whose anti-leishmanial activity was either comparable to or slightly lower than that of amphotericin B, are encouraging and could form the basis of chemical structure optimization to increase anti-leishmanial activity and selectivity.

Molecular docking is a current computational tool employed in the design and discovery of novel drug candidates [53-55]. Classified among the structure-based drug designing approaches, molecular docking studies have been successfully performed to predict, at the atomic level, the experimental binding modes and affinities of small molecules (namely ligands, here represented by the 4-substituted 2-(1H-pyrrolo [3, 2-c] pyridin-2-yl) propan-2-ol compounds) within the binding site of particular receptor targets, herein suggested to be the ADP site of the NDK enzyme. Therefore, molecular docking simulations gain special importance when the putative target is unknown and it may help in the identification and elucidation of the mechanism of action involved in the biochemical process.

The molecular modeling results obtained suggested the possibility that 3, 4, 7 and 11 bind to the ADP site of the LbNDK. Therefore, one may hypothesize that the anti-leishmanial activity observed in the biological testing arises from a competitive inhibition mechanism for these compounds with the natural substrate, ADP. This could interfere in the reversible mechanism of the transfer of a γ-phosphoryl group from a purine triphosphate (NTP) donor to a purine diphosphate (NDP) acceptor as performed by the nucleoside diphosphate kinase, [56] impairing the parasite´s metabolic pathways and possibly leading to parasite death. In agreement with our findings, inhibition of the NDK of *Leishmania major* (LmNDK) by a compound containing a pyrrole-indolinone moiety (a nitrogen containing heterocyclic compound that is similar to 1H-pyrrolo [3, 2-c] pyridines (5-azaindoles)), has been reported during the preparation of this manuscript [9].

Conclusion

A small library of 4-substituted 2-(1*H*-pyrrolo [3, 2-*c*] pyridin-2-yl) propan-2-ols was tested in cell-based assays to discover compounds with novel, broad-spectrum anti-leishmanial activity. While several of the tested compounds displayed anti-leishmanial activity against intracellular amastigotes of one or more *Leishmania* species (*L. infantum*, *L. amazonensis* and *L. braziliensis*), only one of the compounds, **11**, displayed sufficiently selective activity towards *L. infantum*. The molecular modeling study suggested that these compounds are capable of binding to the ADP site of the nucleoside diphosphate kinase from *L. braziliensis*, and, possibly, promoting its inhibition by a competitive mechanism, for example, by interfering in the reversible mechanism that regulates the metabolic pathways of purine nucleotides. Altogether, the data reported here shows that 4-substituted 2-(1*H*-pyrrolo [3, 2-*c*] pyridin-2-yl) propan-2-ols might become promising chemical entities for anti-leishmanial discovery. Future work will focus on improving the synthetic chemistry, which as discussed in our previous report was problematic and limited, [7] and testing for Leishmanial NDK inhibition, in order to evaluate our hypothesis about the molecular mechanism of action, especially, in relation compound 11. In relation to molecular design, consideration should be given to increasing specificity towards the parasite and/or decreasing toxicity to the host cell, establishing structure-activity relationships and drug-likeness properties in order to develop better antiprotozoal candidates.

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