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Computer-aided discovery of two novel chalcone-like compounds active and selective against *Leishmania infantum*

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ABSTRACT

Leishmaniasis are infectious diseases caused by parasites of genus *Leishmania* that affect affects 12 million people in 98 countries mainly in Africa, Asia, and Latin America. Effective treatments for this disease are urgently needed. In this study, we present a computer-aided approach to investigate a set of 32 recently synthesized chalcone and chalcone-like compounds to act as antileishmanial agents. As a result, nine most promising compounds and three potentially inactive compounds were experimentally evaluated against *Leishmania infantum* amastigotes and mammalian cells. Four compounds exhibited EC_{50} in the range of 6.2–10.98 μM. In addition, two compounds, **LabMol-65** and **LabMol-73**, exhibited cytotoxicity in macrophages >50 μM that resulted in better selectivity compared to standard drug amphotericin B. These two compounds also demonstrated low cytotoxicity and high selectivity towards Vero cells. The results of target fishing followed by homology modeling and docking studies suggest that these chalcone compounds could act in *Leishmania* because of their interaction with cysteine proteases, such as procathepsin L. Finally, we have provided structural recommendations for designing new antileishmanial chalcones.

Endemic in 88 countries, leishmaniasis are infectious diseases caused by parasites of genus *Leishmania* and transmitted to the humans by the bite of female phlebotomine sandfly.[1,2] According to the World Health Organization (WHO), around 1.3 million new cases occur per year.[3] Visceral leishmaniasis (VL), also know as Kala-azar, is the most severe form, in which vital organs are affected causing chronic fever, liver issues, spleen enlargement, anemia, and other blood problems.[4,5]

The first-line drugs for treatment of leishmaniasis are the pentavalent antimonials, meglumine antimoniate (Glucantime®) and sodium stibogluconate (Pentosan®). If they fail, second-line drugs such as pentamidine, amphotericin B and miltefosine are used.[2] However, the long-term treatment and severe side effects are limitations of the available drugs. Moreover, resistance development against the available drugs has increased over the years. Additionally, the high cost of some therapies has limited their use for poor people in developing countries. Thus, there is an urgent need for the discovery of new drugs based on new molecular scaffolds for this neglected disease.[4]

Recent advances in genomics have triggered a shift in drug discovery from the paradigm of focusing on strong single-target interaction to more global and comparative analysis of multi-targets network.[6,7] *In silico* methods, including target- and ligand-based strategies, are widely used in industry and academia complementary to experimental techniques.[8] For instance, *in silico* target fishing can enable the discovery of a number of putative targets for a given set of small molecules with known biological effects.[9]

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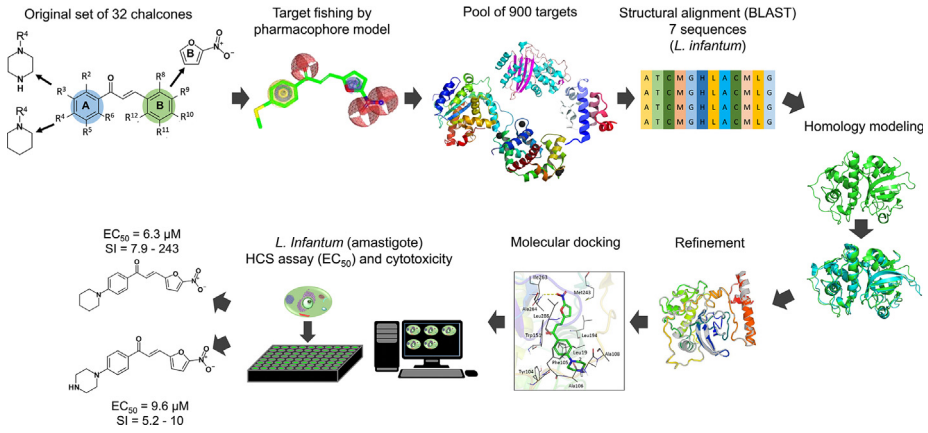


Fig. 1. Computer-aided approach to discovery new chalcones with antileishmanial activity.

Table 1
Results obtained from sequence alignment on BLAST.

Target	Max score	Total score	Query cover	E-value	Identity
Actin	590	590	99%	0.0	70%
Casein Kinase II	400	400	97%	6.00E-143	62%
Cathepsin B	256	256	97%	2.00E-86	45%
Cathepsin L	382	382	86%	4.00E-132	49%
CKdhfr-ts	249	249	99%	1.00E-82	45%
GG3PD	408	408	63%	2.00E-147	85%
Heat stock protein 70	687	687	70%	0.0	73%

Dhfr-ts: Diidrofolate reductase; GG3PD: Glycosomal glyceraldehyde 3-phosphate dehydrogenase.

Table 2
Summary of statistics of obtained 3D models of *L. infantum* proteins.

	Template information			PROCHECK analysis			
Target Uniprot (ID)	Cov.	Seq. Id.	Temp.	MFR	AAR	GAR	DR
Actin (P60010)	99%	72%	1YAG	95.3%	4.3%	0.3%	0.0%
CK2 (P68400)	90%	57%	3PE2	93.7%	6.0%	0.0%	0.4%
CathepsinB (Q6R7Z5)	90%	52%	3MOR	93.3%	5.7%	0.5%	0.5%
Cathepsin L (P07711)	62%	41%	1 CJL	90.4%	9.2%	0.0%	0.4%
Dhfr-ts (A7ASX7)	91%	44%	3NRR	89.1%	9.3%	0.7%	0.9%
G3PD (P22513)	96%	85%	1K3T	92.2%	5.2%	1.6%	1.0%
Hsp70 (P54652)	99%	71%	5FPN	94.7%	5.3%	0.0%	0.0%

CK2: Creatine kinase 2; Cov.: coverage; Seq. Id.: Sequence Identity; Temp.: Template; MFR: Most Favored Regions; AAR: Additional Allowed Regions; GAR: Generously Allowed Regions; DR: Disallowed Regions.

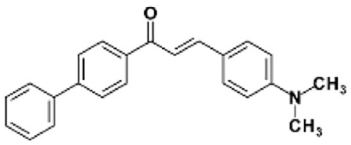
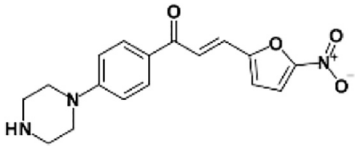
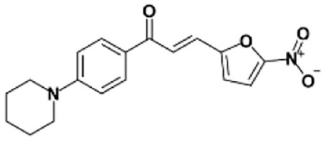
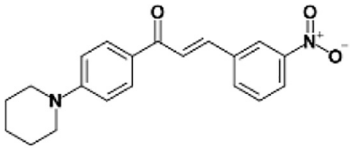
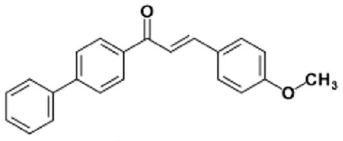
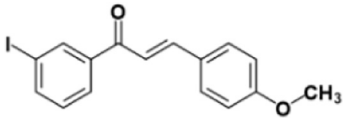
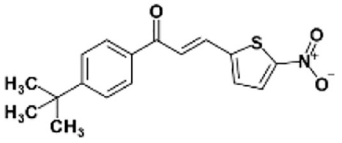
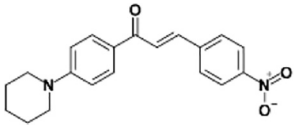
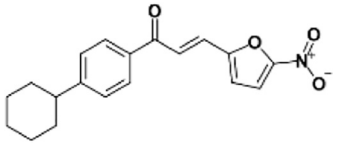
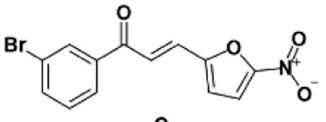
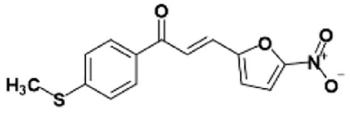
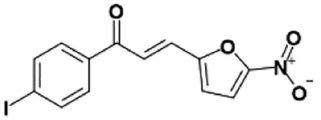
Chalcones are biologically classified as secondary metabolites of low molecular weight. In medicinal chemistry, they are considered privileged structures for research and development of new drugs, due to the diversity of substituents that can be linked to conjugated system scaffold.[10] Chemically, chalcones are classified as 1,3-diaryl-2-propen-1-ones and possess a broad spectrum[11–24] of properties including antileishmanial activity.[25] Previous studies have reported the *in vitro* and *in vivo* activity of chalcones and chalcone-like (heteroaryl chalcone) compounds against *Leishmania donovani*,[26,27] suggesting that chalcones have potential as antileishmanial agents.

The goal of this study was to identify novel antileishmanial compounds among 32 previously synthesized chalcones and heteroaryl chalcones (chalcone-like) compounds.[28] The general workflow is shown in Fig. 1. Initially, 32 compounds have been submitted to a target fishing approach using pharmacophore modelling. Then, the 3D structures of selected targets were obtained by homology modeling and we performed molecular docking with the 32 chalcones and the selected targets. Finally, the *in vitro* biological activity on *Leishmania infantum*, cytotoxicity on macrophages and Vero cells and selectivity of promising compounds were evaluated experimentally.

On the search of potential targets for the antileishmanial hits, we used the PharmMapper server,[7,29] a database that is backed up by a large, in-house repertoire of pharmacophore information extracted from all the targets available in TargetBank, DrugBank, BindingDB, and PDTD (Potential Drug Target Database). The original dataset of 32 chalcones and chalcones-like compounds was submitted to the web server, generating a list of targets and a maximum of 300 conformations for each ligand, which were ranked by the fit score to the pharmacophore model. These results are presented on Table S1 (Supporting Information). Then, all targets were aligned on BLAST server.[30] As a result, 7 sequences were identified as potential targets for *L. infantum* hits (Table 1), all presenting high primary sequence identity (>30%).

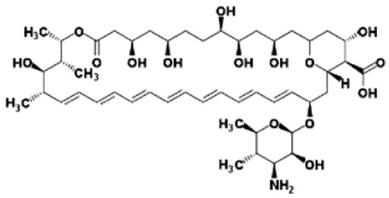
Based on these results, homology models of the seven selected proteins were built on SWISS-MODEL server[31] (Table 2), by comparing target sequences with sequences of other proteins with available 3D structures, which were used as templates. The quality of the models was evaluated in PROCHECK[32], and the quality of dihedral angles

Table 3
In vitro antileishmanial activity **EC_{50} (μM)**, toxicity (**CC_{50} μM)** and selectivity index (SI) in macrophages and Vero cells of chalcones and chalcones-like.

Code	Structure	EC_{50} (μM)	*CC_{50} (μM)	*SI	**CC_{50} (μM)	**SI
LabMol-69		>50	>50	N.D	N.D	N.D
LabMol-73		9.6	>50	>5.2	>100	10
LabMol-65		6.3	>50	>7.9	349	55
LabMol-67		30.7	>50	>1.6	N.D	N.D
LabMol-70		>50	>50	N.D	N.D	N.D
LabMol-76		>50	>50	N.D	>100	2
LabMol-86		31.07	8.4	0.2	>100	3
LabMol-90		>50	>50	N.D	N.D	N.D
LabMol-72		10.9	31.1	2.8	>100	10
LabMol-82		*23.8*	*14.9*	*0.6*	*68.8*	*2.9*
LabMol-92		*9.3*	*13.1*	*1.4*	*40.7*	*4.3*
LabMol-78		*27.1*	*49.5*	*1.8*	*>100*	*3.6*

(*continued on next page*)

Table 3 (*continued*)

Code	Structure	EC_{50} (μM)	*CC_{50} (μM)	*SI	**CC_{50} (μM)	**SI
Amph.B		1.9	9.8	>5.2	N.D	N.D

* Macrophage.
** Vero cells.

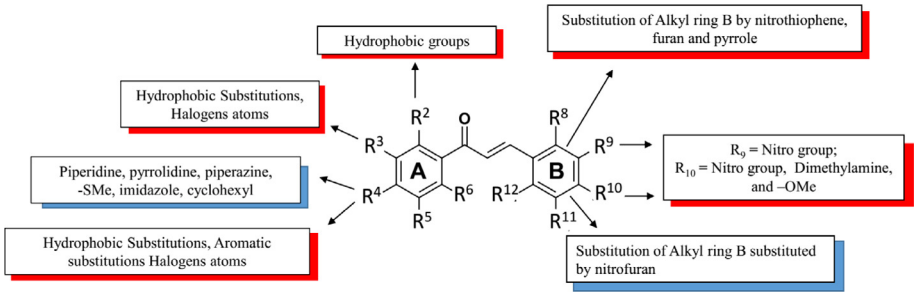


Fig. 2. Derived SAR rules highlighting structural moieties favorable and unfavorable to the anti-leishmanial activity. Red boxes are unfavorable groups and blue boxes are favorable groups.

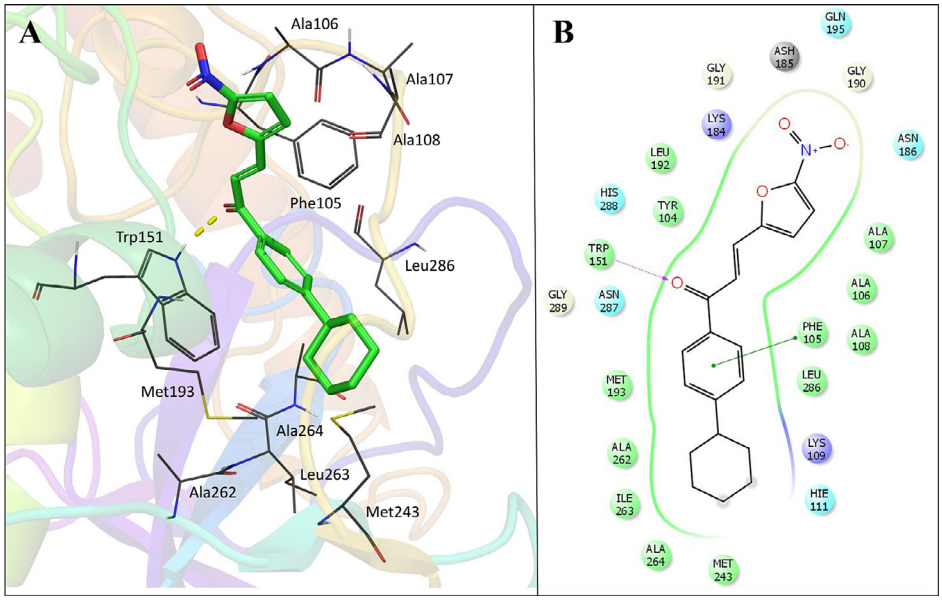


Fig. 3. 3D (**A**) and 2D (**B**) visualization of interactions of **LabMol-72** within the binding site of procathepsin L, obtained by docking.

(phi and psi) was analyzed. Furthermore, GalaxyWEB[33] was used to refine loop and terminus regions of the best template of each target. The results are presented on Table 2 and Supplementary Fig. S1 (A-G). It can be observed that 89.1–94.7% of residues from the modeled proteins are on the most favored regions (red), 5.2–9.3% on the allowed regions (yellow), 0.0–1.6% on the generously allowed regions (beige) and just 0.0–1.0% on the disallowed regions (white).

The residues in the disallowed regions were located in regions far from the binding sites, and therefore, did not affect the quality of the models. Therefore, the generated homology models could be used for the estimation of the binding modes and affinity of ligands to the proteins by docking.

After the building, selection, and analysis of the homology models, they were used to perform molecular docking of chalcone and chalcone-like compounds. Chemical structures were carefully curated following the protocols developed by Fourches et al.[34–36] Based on the results of docking (Supplemental Table S2), we have selected nine promising compounds (**LabMol-69**, **73**, **65**, **67**, **70**, **76**, **86**, **90**, and **72**) and potentially inactive compounds (**LabMol-82**, **92**, and **78**) as negative controls.

Twelve selected chalcone-like compounds and amphotericin B, used as positive control, were tested against *L. infantum* amastigotes and differentiated THP-1 macrophages (Table 3). Three out of nine selected compounds (**LabMol-65**, **LabMol-72**,

and **LabMol-73**) showed reasonably high activity ($6.32 < EC_{50} < 10.98$ μM). Other six compounds were inactive. Amphotericin B exhibits EC_{50} of 1.9 μM. Among negative controls, **LabMol-72**, and **LabMol-73** were expectedly inactive, while **LabMol-92** has demonstrated EC_{50} of 9.31 μM. Among four active compounds, during cytotoxicity assays on macrophages, only **LabMol-65** and **Labmol-73** showed $CC_{50} > 50$ μM that resulted in SI > 5.2 and 7.9, respectively (Table 3). Amphotericin B exhibits CC_{50} of 9.8 μM and SI of 5.2; therefore, **LabMol-65** exhibited SI higher than the control and **Labmol-73** showed selectivity index (SI) similar to or higher than the control. All active compounds were also tested against Vero cells[28] (Table 3). **LabMol-65** and **Labmol-73** demonstrated SI's in the range 55–243 and 4–10, respectively, that make them promising anti-leishmanial agents. All the details regarding conducted experiments and the complete table with biologic evaluation (Table S3) are available in Supplemental Information.

Based on the experimental results, we derived structure-activity relationships (SAR) rules to reveal the structural fragments responsible for antileishmanial activity (Fig. 2). On the aryl ring B, independently of substituent positions on ring A, nitro group in position R^9 and nitro-, dimethylamino-, and methoxy- groups in position R^{10} decrease the activity. The substitution of aryl ring B by furan and 5-nitrothiophene are also unfavorable. However, the substitution of aryl ring B by 5-nitrofuran is favorable to biological activity. Bulky groups and electron donor groups on R^4 position of aryl ring A, e.g., piperidine, pyrrolidine, piperazine, methylthiole, imidazole, and cyclohexyl are favorable for antileishmanial activity, while methyl, *t*-buthyl, buthyl, phenyl, morpholine and halogens atoms are unfavorable. The hydrophobic substituents and halogen atoms tested in positions R^2 and R^3 also demonstrated negative contribution to antileishmanial activity.

The results of target fishing approach, followed by homology modeling and molecular docking allowed us to rationalize the mode of action of four active compounds (**LabMol-65**, **LabMol-72**, **LabMol-73**, and **LabMol-92**). They might interact with the cysteine protease procathepsin L, demonstrating their potential for blocking the replication and differentiation of *Leishmania in vitro* and *in vivo*. These analyses revealed that the exploration of modifications on scaffolds of chalcones identified here could afford new promising candidates against *L. infantum* and suggest that the mode of action of these compounds could be by inhibition of cysteine proteases of the parasite.

Cysteine proteases constitute an important class of enzymes responsible for virulence factors, essential to parasite survival and are potential drug targets.[37–39] Fig. 3A and B show the obtained docked poses for **LabMol-72** and its molecular interactions in the active site of procathepsin L. As we can see, hydrophobic interactions and the hydrogen bond are showed. The analysis of the hits in the active site cavity reveals that the hydrophobic pocket is important for interaction with procathepsin L. Moreover, Trp151 plays a significant role by performing a hydrogen bond with the carboxyl group of chalcone (see SI Figs. S2, S3, S4 and Table S4 on Supplementary data).

To summarize, the set of 32 recently synthesized[28] chalcone and chalcone-like compounds was evaluated by computational approaches to verify their potential antileishmanial activity. By results of this *in silico* evaluation, nine potentially active and three potentially inactive compounds were experimentally tested against *L. infantum* amastigotes. Four compounds showed $EC_{50} < 11$ μM. Among them, two compounds, **LabMol-65** and **LabMol-73**, exhibited cytotoxicity in macrophages > 50 μM that resulted in better selectivity than the standard drug amphotericin B. These two compounds also demonstrated low cytotoxicity and high selectivity towards Vero cells. Based on modeling results, we suggested that activity of these compounds is caused by their interaction with cysteine proteases of the parasite. We also conducted SAR analysis to derive structural recommendations useful for molecular design of new chalcones or chalcone-like compounds with antileishmanial activity. For instance, the substitution of aryl ring B by 5-nitrofuran is favorable. The other nitrofuran analogues, nitrothiophenes, aromatic rings, pyrrole, and furan analogues were inactive against amastigotes of *L. infantum* (see Supplementary Table S3). These results corroborates with other studies which demonstrated that chalcones[5,25] and nitroheterocycle[40] compounds are active against *Leishmania* species.

Conflict of interest

The authors declare no competing financial interest.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.04.010.

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