

# In-Silico Identification of the Best Compound Against *Leishmania infantum*: High Throughput Screening of All FDA Approved Drugs

*Leishmania infantum*'a Karşı En İyi Bileşimin Silico Olarak Tanımlanması: Tüm FDA Onaylı İlaçların Yüksek Verimli Tarama

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## ABSTRACT

**Objective:** Current in-silico research was designed and administered for the screening of 20000 Food and Drug Administration-approved drug compounds with the goal of finding promising drugs against lipophosphoglycan (LPG) and  $\gamma$ -glutamylcysteine synthetase ( $\gamma$ -GCS) of *Leishmania infantum*.

**Methods:** After the protein sequence of both targets was taken, the 3D structures of protein of interest were predicted and validated. Molecular docking was done among the two putative targets (LPG and  $\gamma$ -GCS) and approved compounds were selected using AutoDock 4.2 program to predict ligand-receptor interactions.

**Results:** After docking experiment was done on 20000 drug compounds, a total number of seven ligands, two for  $\gamma$ -GCS receptor and five for LPG receptor, were assigned as novel, potent anti-leishmanial drugs based on their binding affinity and energy. Of those, five ligands possessed cytotoxic and anti-cancer characteristics and showed good binding capacity to LPG receptor with  $\Delta_{\text{Gbinding}}$  up to 8.5 kcal/mol more negative; while two compounds showed good binding capacity to glutamyl receptor with  $\Delta_{\text{Gbinding}}$  up to 7.8 kcal/mol more negative.

**Conclusion:** The latest software-based methods are powerful tools for scanning and predicting new peptide templates specific to biological targets in organisms for new drug discovery. However, the use of *in vitro* and *in vivo* techniques is a requirement for better evaluation of the potential of projected ligands with the help of in-silico approaches, identifying molecular mechanism of action of the more active compounds is possible. This can help in defining the most likely molecular target, so that the subsequent optimization using *in vitro* and *in vivo* techniques can be undertaken.

**Keywords:** In-silico, lipophosphoglycan,  $\gamma$ -glutamylcysteine synthetase, molecular docking, cytotoxic and anti-cancer activity

## ÖZ

**Amaç:** Mevcut in-silico araştırması, *Leishmania infantum* lipofosfoglikan (LPG) ve  $\gamma$ -glutamilsistein sentetazına ( $\gamma$ -GCS) karşı umut verici ilaçlar bulmak amacıyla 20000 Gıda ve İlaç İdaresi onaylı ilaç bileşiklerinin taranması için tasarlandı ve uygulandı.

**Yöntemler:** Her iki hedefin protein sekansı alındıktan sonra, 3D yapıları tahmin edildi ve doğrulandı. Moleküler yerleştirme iki varsayılan hedef (LPG ve  $\gamma$ -GCS) arasında yapıldı ve ligand-reseptör etkileşimlerini tahmin etmek için AutoDock 4.2 programı kullanılarak onaylanmış bileşikler seçildi.

**Bulgular:** Yirmi bin ilaç bileşiği üzerinde deney yapıldıktan sonra,  $\gamma$ -GCS reseptörü için iki ve LPG reseptörü için beş olmak üzere toplam yedi ligand, bağlanma afiniteleri ve enerjilerine göre yeni, güçlü anti-leishmanial ilaçlar olarak belirlenmiştir. Bunlardan 5 ligand 8,5 kcal/mol'e kadar daha negatif  $\Delta_{\text{Gbinding}}$  ile LPG reseptörüne iyi bağlanma kapasitesi gösteren sitotoksik ve anti-kanser özelliklere sahipti. Bunlardan 2 ligand, 7,8 kcal/mol'e kadar daha negatif  $\Delta_{\text{Gbinding}}$  ile glutamyl reseptörüne iyi bir bağlanma kapasitesi gösterdi.

**Sonuç:** En yeni yazılım tabanlı yöntemler, yeni ilaç keşfi için organizmalarda biyolojik hedeflere özgü yeni peptid şablonlarını taramak ve tahmin etmek için güçlü araçlardır. Bununla birlikte, *in vitro* ve *in vivo* tekniklerin kullanımı, öngörülen ligandların



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potansiyelinin daha iyi değerlendirilmesi için bir gerekliliktir. İn-silico yaklaşımların yardımı ile daha aktif bileşiklerin moleküler düzeyde etki mekanizmasının tanımlanması mümkün olmaktadır ve böylece en olası moleküler hedef belirlenebilmekte ve *in vitro* ve *in vivo* teknikleri kullanılarak sonraki optimizasyon yapılabilmektedir.

**Anahtar Kelimeler:** İn-siliko, lipofosfoglikan,  $\gamma$ -glutamilsistein sentetaz, moleküler yerleştirme, sitotoksik ve anti-kanser aktivitesi

## INTRODUCTION

The protozoan parasites of *Leishmania* spp. are the causative agents of a neglected disease called leishmaniasis, being considered as a significant health concern in tropical and subtropical regions of the world (1). This infection usually disseminates via the bite of a female sand fly and renders different clinical manifestations from cutaneous ulcers to mostly pernicious visceral leishmaniasis (VL) (2). Approximately, one hundred nations have documented leishmaniasis infection with 350 million at-risk individuals residing in endemic territories. As a global perspective, 12 million people are infected and the incidence rates for VL is calculated to be 0.2-0.4 million cases annually (3). Over ninety percent of VL cases occur in six countries, including Brazil, India, Sudan, Bangladesh, South Sudan and Ethiopia (4). Multiple factors are involved in the incidence of leishmaniasis, comprising sandfly and reservoir control issues, increased traditional traveling, suppressed host immunity due to disease complications and some drugs, lack of efficacious immunoprophylaxis as well as drug resistance to traditional therapies (5,6). It has been disclosed that currently administered anti-leishmanial drugs are costly and may possess toxicity and develop drug resistance; hence, it is an urgent need for the development of novel, safe and affordable drugs to combat leishmaniasis (7,8). Approved drugs, especially those approved by Food and Drug Administration (FDA), have favorable or validated pharmacokinetic properties and toxicological profiles, and the repositioning of existing drugs for new indications can potentially avoid expensive costs associated with early-stage testing of the hit compounds (9). The fundamentals of drug discovery rely on the determination of a distinct protein and/or metabolic pathway specific to the pathogen (10). Bioinformatics is a computer-based interdisciplinary approach for various inter-molecular prediction purposes such as peptide-receptor interactions (11). Recognizing structure-activity associations using in-silico screening would efficiently lead us to identify specific drug targets for such neglected infections (12). The conventional drug discovery needs about 14 year and approximately 2.5 billion USD to approve and launch a new drug. To overcome time and cost obstacles to conventional drug discovery the process of assessing new indication for existing and approved drugs called drug repositioning (DR), has been introduced so far. Through taking advantage of drugs already in clinical use for other indications or drugs that have cleared early safety phases of trials, DR can reduce the time and cost of the drug discovery process. Several in-silico approaches have been established to apply the DR, including omics-based and molecular docking based approaches.

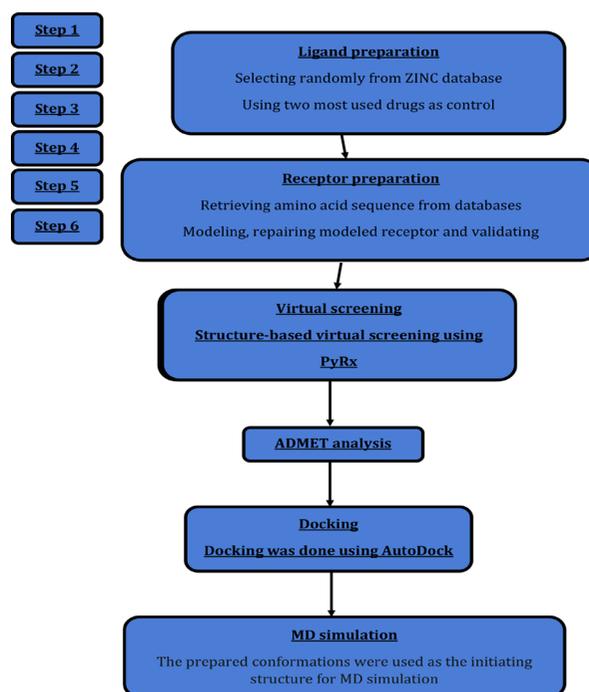
Trypanothione is a crucial molecule in the initial confront with host macrophages, neutralizing their reactive oxidative species and redox potential maintenance (13,14). During its biosynthesis, the enzyme  $\gamma$ -glutamylcysteine synthetase ( $\gamma$ -GCS) catalyses the first and rate-limiting phase and possess imperative action in parasite survival, rendering it as a considerable drug target (15,16). In contrast to other trypanosomatid species, the glycocalyx of *Leishmania* parasites constitutes of phosphoglycosylated glycans anchors of glycosylphosphatidylinositol (GPI). One of

the most outstanding glycoconjugates of the *Leishmania* surface membrane is lipophosphoglycan (LPG) (17). This molecule may be an appropriate drug target since it is a strong agonist of toll-like receptors, inhibits the activation of protein kinase C, harnesses complement system and damages phagolysosome maturation (18). In this experimental study, we aimed to investigate in-silico anti-leishmanial activity of 3358 FDA-approved compounds against above-mentioned drug targets of *Leishmania infantum* (*L. infantum*) and compared to amphotericin B and glucantime.

## METHODS

### Template Selection and Confirmation

At first, the FASTA format of amino acids sequences of LPG (Uniprot ID: A4I4C9) and  $\gamma$ -GCS (Uniprot ID: A4HY37) for *L. infantum* were gathered from the UniProt Database (<https://www.uniprot.org>), and was used in BLAST analysis. Then, a program search protein databases using a protein query called BLASTP was run against the protein data bank to find a three-dimensional (3D) determined structure according to the best alignment scores. Subsequently, respective 3D structural models for both molecules were created using Swiss-Model online software (<https://swissmodel.expasy.org>), then validated by Protein Model Portal (<https://www.proteinmodelportal.org>), saves v5.0 (<http://servicesn.mbi.ucla.edu/SAVES/>), and Protein Structure Evaluation Suite & Server (<http://www.prosess.ca>) web servers.



**Figure 1.** Schematic workflow for finding potential drug, ligands and receptors specific to the *L. infantum* receptor

Finally, the selected protein was simulated using GROMACS 5.1.3 software (19) for 100 ns base on the ABMER03 force field. The flow diagram of finding potential drug, ligands and receptors specific to the *L. infantum* receptor has given in details (Figure 1).

### Ligands Library Preparation

We used a method for template and FDA approved drug selection that we conducted and published previously (20). In brief, 20000 FDA approved drugs were randomly extracted from the ZINC database and tested against two selected receptors. For comparative study, the 3D structure of two known anti-leishmanial drugs from Drug Bank (<https://www.drugbank.ca>) and PubChem (<https://pubchem.ncbi.nlm.nih.gov>) databases were screened and appointed. Thus, a library of potential ligands was built. Also, two drug targets and FDA-approved drug compounds were prepared for molecular docking. All ligands in the pdbqt format were retrieved using openbabel software and their energy was minimized by MMFF94 force field.

### Virtual Screening, and Control Setting

In this study, we used structure-based virtual screening, a computational-aided technique used in the early-stage drug discovery campaign to search a chemical compound library for novel bioactive molecules against a drug target of interest (21). We used PyRx Software for virtual screening procedure. All 20000 selected FDA-approved drugs were docked using AutoDock Vina in PyRx 0.8 (22). These drugs with their chemical structure, physical properties, and clinical indications have been retrieved from various public databases, including PubChem, CheMBL, DrugBank, and DrugCentral (23). Glucantime and amphotericin B were used as control drugs in our experiment because they are standard treatment options for VL.

### Molecular Docking

The AutoGrid section of AutoDock tools was employed to compute the grid maps of all LPG and  $\gamma$ -GCS molecules, taking into account large enough ones to include surface and active site. AutoDock 4.3 software was used for docking the chosen drug compounds into the active site of LPG and  $\gamma$ -GCS. The Lamarckian genetic algorithm-based AutoDock 4.2 was applied for ligand-receptor interactions. To increase docking efficiency, the total number of 63800 runs were performed for each drug target. Upon yielding docking values, cluster analysis was fulfilled in accordance with the root mean square (0.5A).

The ligands were sketched and geometrically minimized using ACD ChemsSketch ([www.acdlabs.com](http://www.acdlabs.com)) and Marvin Sketch ([www.chemaxon.com](http://www.chemaxon.com)), respectively. The ligands and proteins were then prepared using AutoDock Tools 1.5.6 ([www.autodock.scripps.edu](http://www.autodock.scripps.edu)). The proteins were added with polar hydrogen and given by Kollman charge, whereas the ligands were given by Gasteiger charges. The grid was centered on each protein binding site and the docking was then performed using AutoDock Vina embedded in PyRx version 8.0 ([www.autodock.scripps.edu](http://www.autodock.scripps.edu)). The hit of protein was selected based on the lowest mean of free energy of binding ( $D G_{\text{bind}}$ ), whereas a combination of a low  $D G_{\text{bind}}$  and hydrogen bond interaction was considered to select the hit of the ligand. The selected docking pose was visualized using discovery studio 3.5 ([www.accelrys.com](http://www.accelrys.com)).

In the following, AutoDock Vina and AutoDock 4.2 were assigned to calculate free energy charge of binding and binding affinity. Below equation corresponds to computation of estimated free energy charge of binding: (6)

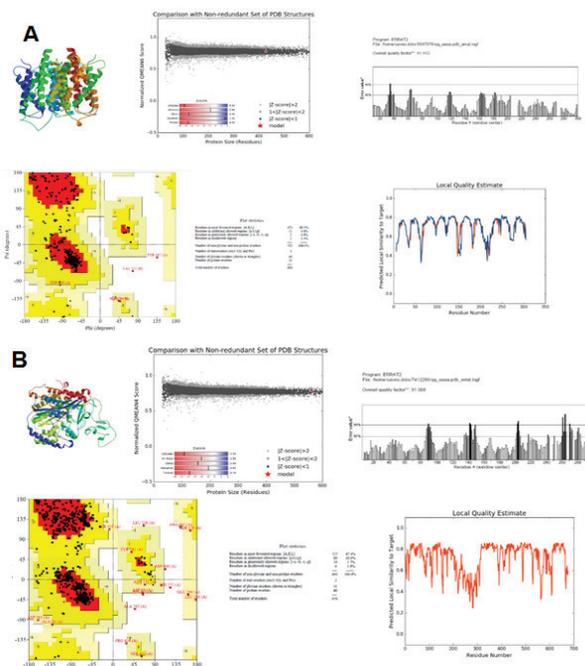
estimated free energy charge of binding = torsional free energy of ligand + final inter - molecular energy

### Molecular Dynamic (MD) Simulation

After preparing the template and ligands, the prepared conformations were used as the initiating structure for molecular dynamic (MD) simulation. The acquired conformation of MD, as well as the docked ligands, were engaged in the MD simulation process. All MD simulations were done using the GROMACS 5.1.3 software package and the AMBER03 force field, in a box with dimensions of 96×95×100 Å. The force field parameters were acquired from the antechamber module (22) of the Amber program, using the GAFF force field (23). Partial charges of the ligands were assigned using the AM1-BCC model (24), and an energy minimization method was performed. After energy minimization, a position restraint process was accomplished in association with NVT and NPT ensembles. Finally, the selected protein was simulated using GROMACS 5.1.3 software (19) for 100 ns base on the ABMER03 force field.

## RESULTS

In total, three and seven templates were predicted using Swiss-model for LPG and  $\gamma$ -GCS, respectively. The best model for LPG was the second model, whereas the best one for  $\gamma$ -GCS was the first template, with XP\_001468657.1 vs XP\_001464978.1 sequence identities, e-values of 1.18962e-124 vs 2.26415e-140 and final total model energies of -12670.841 kJ/mol vs -12892.576 kJ/mol (Figure 2A and 2B).

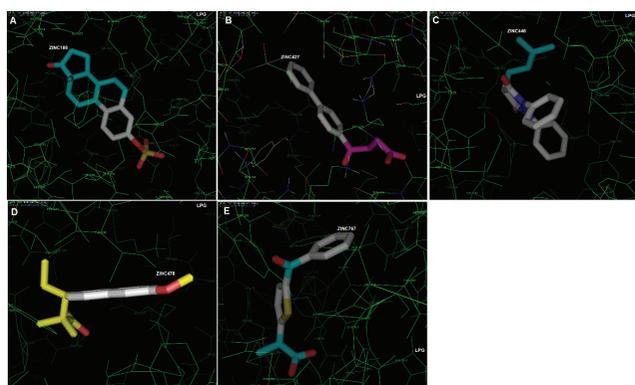


**Figure 2.** A) The LPG model from *L. infantum* using homology modeling software, B) The  $\gamma$ -GCS model from *L. infantum* using homology modeling software. The upper, left figure shows the prepared model for the receptor of interest, the upper, right figure shows model validation with ERRAT, the bottom left figure shows Ramachandran plot with the percentage of amino acids in favored and allowed regions, and the bottom, right figure shows the detail of model local quality

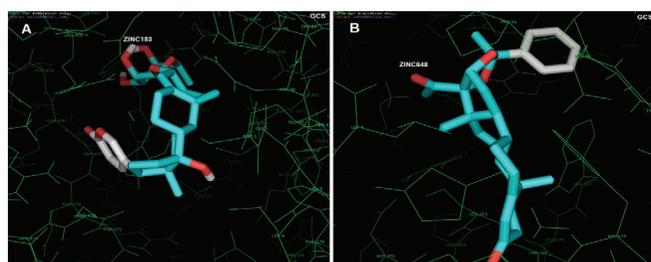
Model validation was also done and Ramachandran plots were calculated for LPG and  $\gamma$ -GCS, suggesting 99.1% vs. 97.4% residues in the allowed region, respectively. According to ERRAT program results, the total quality factors of provided models for LPG and  $\gamma$ -GCS were as 91.912 and 91.008, respectively. Chemical properties of selected FDA-approved compounds were given in detail in Table 1.

After the accomplishment of docking on 20000 drug compounds, a total number of seven ligands, two for  $\gamma$ -GCS receptor and five for LPG receptor, were assigned as novel, potent anti-leishmanial drugs based on their binding affinity and energy (Table 2).

Of these, five ligands possessed cytotoxic and anti-cancer characteristics that showed a good binding capacity to LPG receptor with  $\Delta G_{\text{binding}}$  up to 8.5 kcal/mol more negative (Figure 2A, C, D, E); while, two compounds showed good binding capacity to Glutamyl receptor with  $\Delta G_{\text{binding}}$  up to 7.8 kcal/mol more negative (Figure 3A and B). The selected compound 186 inserted in the hydrophobic pocket created by Met237, Val241, Ser254, and Gly260, as shown in Figure 2A. The compound 427 inserted in the hydrophobic pocket created by Thr104, Lys107, Met237, Thr238, and Val241, as shown in Figure 2B. The compound 446



**Figure 3.** A) Experimental ligand no. 186 (zinc186) that showed H-binding to LPG receptor, B) experimental ligand no. 427 (ZINC427) that showed H-binding to lipophosphoglycan (LPG) receptor C) experimental ligand no.446 (ZINC446) forms H-bond with LPG receptor, D) experimental ligand no.478 (ZINC478) forms H-bonds with LPG receptor, E) Experimental ligand no.767 (ZINC767) that showed H-binding to LPG receptor form *L.infantum*. The selected compound 183 inserted in the hydrophobic pocket created by Gly321, Val381, Ser474, Gln479, and Arg498, as shown in Figure 3A. The compound 648 inserted in the hydrophobic pocket created by Pro91, Leu189, Glu192, Ser474, and Trp477, as shown in Figure 3B.



**Figure 3.** A) Experimental ligand no. 183 (zinc183) that showed H-binding to  $\gamma$ -GCS receptor, B) experimental ligand no. 648 (ZINC648) that showed H-binding to  $\gamma$ - GCS receptor form *L. infantum*

GCS: Glutamylcysteine synthetase

**Table 1.** Chemical characteristics of selected FDA-approved drugs

Ligand	pH range	xlogP	Apolar desolvation (kcal/mol)	Polar desolvation (kcal/ml-L)	H-bond donors	H-bond acceptors	Net charge	tPSA (Å <sup>2</sup> )	Molecular weight (g/mol)	Rotatable bonds	Heavy atoms
ZINC08101183	Reference (pH 7)	2.72	-5.96	-18.25	4	8	0	129	530.658	3	38
ZINC03830648	Reference (pH 7)	5.05	2.14	-9.61	0	4	0	52	48.603	2	33
ZINC00000767	Reference (pH 7)	3.28	8.84	-44.43	0	3	-1	57	259.306	4	18
ZINC00001427	Reference (pH 7)	3.04	9.57	-46.3	0	3	-1	57	253.277	5	19
ZINC00001446	Reference (pH 7)	4.41	9.04	-53.1	0	4	-1	50	319.384	4	24
ZINC03876186	Reference (pH 7)	0.84	6.27	-49.4	0	5	-1	84	349.428	2	24
ZINC00000478	Reference (pH 7)	4.88	2.45	-51.68	0	3	-1	49	285.363	5	21

FDA: Food and drug administration

Table 2. Report of potential anti-leishmanial new drugs using docking results

Ligand	Binding energy	Δ G	Binding affinity	Drug name	PubChem CID	MW (g/mol)	MF	Safety summary	Use
<b>Glutamyl receptor</b>									
ZINC08101183	-4.73	-7.83	-9.5	Proscillaridin	CID 4951	530.658	C <sub>30</sub> H <sub>42</sub> O <sub>8</sub>	-----	Potential cytotoxic and anti-cancer property
ZINC03830648	-6.49	-7.28	-8.9	Deladroxone	CID 5284538	448.603	C <sub>23</sub> H <sub>36</sub> O <sub>4</sub>	-----	Anti-inflammatory
<b>LpG receptor</b>									
ZINC00000767	-8.13	-8.49	-7.2	Tiaprofenic acid	CID 190591	260.307	C <sub>14</sub> H <sub>12</sub> O <sub>3</sub> S	-----	A non-steroidal anti-inflammatory drug
ZINC00001427	-7.61	-8.02	-7.9	Fenbufen	CID 3335	254.285	C <sub>16</sub> H <sub>14</sub> O <sub>3</sub>	-----	A non-steroidal anti-inflammatory drug
ZINC00001446	-7.08	-8.48	-8.4	Feprazone	CID 35455	320.392	C <sub>20</sub> H <sub>20</sub> N <sub>2</sub> O <sub>2</sub>	-----	Anti-inflammatory,
ZINC03876186	-7.72	-7.88	-7.8	Estrone sulfate	CID 3001028	350.429	C <sub>18</sub> H <sub>22</sub> O <sub>5</sub> S	-----	Potential cytotoxic and anticancer property
ZINC00000478	-7.71	-7.86	-8.0	Methallenestril	CID 10599	286.371	C <sub>18</sub> H <sub>22</sub> O <sub>3</sub>	-----	Potential cytotoxic and anticancer property
<b>Control drugs for glutamyl receptor</b>									
Amphotericin B	-6.91	283.77	-12.8	Amphotericin B	CID 5280965	924.091 g/mol	C <sub>47</sub> H <sub>73</sub> NO <sub>17</sub>	-----	Macrolide antifungal antibiotic
Meglumine antimonate	-4.32	223.76	-4.5	Meglumine antimonate	CID 64953	365.98 g/mol	C <sub>7</sub> H <sub>18</sub> NO <sub>6</sub> Sb	-----	Antimony salt of meglumine that is used in the treatment of leishmaniasis
<b>Control drugs for LPG receptor</b>									
Amphotericin B	-5.64	279.25	-11.4	Amphotericin B	CID 5280965	924.091 g/mol	C <sub>47</sub> H <sub>73</sub> NO <sub>17</sub>	-----	Macrolide antifungal antibiotic
Meglumine antimonate	-3.93	241.15	-3.9	Meglumine antimonate	CID 64953	365.98 g/mol	C <sub>7</sub> H <sub>18</sub> NO <sub>6</sub> Sb	-----	Antimony salt of meglumine that is used in the treatment of leishmaniasis

\* MW: Molecular weight, MF: Molecular formula

inserted in the hydrophobic pocket created by Val111, Thr166, Tyr169, Met253, and Gly257, as shown in Figure 2C. The compound 478 inserted in the hydrophobic pocket created by Thr104, Lys107, Ala110, Ser254, and Gly257, as shown in Figure 2D. The compound 767 inserted in the hydrophobic pocket created by Thr48, Leu82, Met92, Leu195, and Asn260, as shown in Figure 2E.

## DISCUSSION

The routine medications used against VL usually entail cytotoxicity, discomfort, and development of resistant strains (7,24-26). Hence, peptides with various metabolic activity may be considered as potent vaccine candidates and drug targets (27,28). During the invasion, *Leishmania* expresses a wide spectrum of proteins with different metabolic and/or pathogenic activity (29,30). A number of such proteins have been discerned as drug targets for modern leishmaniasis treatment, including dihydrofolate reductase, pteridine reductase enzymes, protein kinases and topoisomerases (6,31,32). With respect to the function of  $\gamma$ -GCS and parasite survival (15,18), we selected them as robust drug targets in our investigation, which hadn't been assessed previously. As the most plentiful GPI-anchored molecule of *Leishmania* promastigotes, LPG has been proved to be central to complement inhibition, macrophage entrance, transformation to amastigote form and tolerance to hydrolytic and oxidant circumstances (33). The thiol redox metabolism is one of the outstanding genetic hallmarks of *Leishmania* (34). Trypanothione [T (SH)<sub>2</sub>] system constitutes a considerable part of reduced thiols, which equips the parasite to withstand drugs with antimonial elements (35-37). The first step of T (SH)<sub>2</sub> biosynthesis is catalyzed by  $\gamma$ -GCS enzyme, which has also been identified in an evolutionary-related organism, *Trypanosoma brucei* (*T. Brucei*) (34,38). Wild resistant isolates (39) or cultivated *Leishmania* parasites (40) have been shown to overexpress or amplify the  $\gamma$ -GCS gene. Furthermore, RNAi knock-down of the respective gene resulted in cell death of *T. brucei* (41). These findings

highlight the significance of  $\gamma$ -GCS, the important rate-limiting catalyzer of glutathione biosynthesis, as a compelling drug target for modern leishmaniasis treatment options.

The “*in-silico*” analysis which stands for computer-based biological experiments, is a state-of-the-art and accurate method to discover exclusive bioactive compounds, which may represent novel metabolic pathways and/or a powerful affinity to a certain target (42). In this sense, several studies have identified unprecedented molecules for various drug targets in *Leishmania*, such as pteridine reductase1, trypanredoxin peroxidase and sterol biosynthesis (43-48), as well as in other single-celled eukaryotes enclosing *Toxoplasma gondii*, *Cryptosporidium hominis*, *Plasmodium* and *Trypanosoma cruzi* (49-51). Current *in-silico* investigation was aimed to screen and predict the anti-leishmanial potency of 3358 FDA-approved compounds against both drug targets in *L. infantum* in comparison to amphotericin B and glucantime for the discovery of new biochemical molecules. Using computer-aided tools, only seven out of 3358 tested compounds were qualified to be a biological ligand for our targets, three with anti-tumor activity and the others with anti-inflammatory features. Approving the antiprotozoal activity of certain compounds with anti-cancer or anti-inflammatory properties is one of the significant advances. The putative cytotoxicity induced by anti-cancer compounds in *Leishmania* may arise from the truth that both tumor cells and parasites assign biochemical analogies, which impact DNA and polyamine metabolism, glucose catabolism and the pathways of protein kinases (52). Similar to apoptosis pathway of multicellular livings, a fascinating mechanism of programmed cell death has also been recognized in protists such as *Leishmania* spp., which could be a rational explanation for outlandish anti-leishmanial effects of some antitumor substrates (53,54). So far, a number of anticancer drugs with various mechanisms of action have been tested for the aim of leishmaniasis treatment *in vitro* and *in vivo*, including doxorubicin (interaction with DNA), aniline-acrydines (topoisomerase II inhibition), phospholipid analogues (lipid metabolism inhibition and cell membrane destruction), 8-hydroxyquinolines (inhibition of electron transfer chain) and sunitinib (tyrosine kinase inhibitor) (32). Among these, tamoxifen, raloxifene, imiquimod, imatinib and sunitinib possessed good results in leishmaniasis animal models and are appropriate candidates for further evaluations (32). The efficacy of some anticancer protein kinase inhibitors was evaluated by Sanderson et al. (55), showing sarafenib, lapatinib and sunitinib as bioactive compounds against *L. donovani* amastigotes in murine macrophages. In the context of anti-leishmanial activity of anti-inflammatory drugs, few compounds have been examined and less is understood regarding how they would impress the parasite molecular targets. For instance, murine systematic leishmaniasis was shown to be inhibited by acetyl salicylic acid-induced nitric oxide immunomodulation (56); however, this finding doesn't indicate a ligand-target interplay. Thus, the impact of anti-inflammatory drugs on potent *Leishmania* drug targets requires further *in-silico* and *in vitro* excavation and is still open to question.

In total, high-throughput computer-aided analyses showed us a number of anti-inflammatory and anti-cancer compounds with anti-leishmanial activity, according to their well-established interaction to the respective parasite ligands ( $\gamma$ -GCS and LPG). However awaits *in vitro*, *in vivo* and clinical validations, such repurposed compounds would inhibit the parasite survival more efficiently than control drugs, as confirmed by *in-silico* investigations and obviates the need for current costly drugs with significant side effects.

## CONCLUSION

With the advent of the computer era, software-based tools have paid a huge contribution in accurate predictions, comprising epitope mapping, drug-target interactions, etc. This cutting-edge method helps us to screen and predict novel peptide templates specific to biological targets in organisms leading to novel drug discovery. The present study demonstrated seven potent antileishmanial ligands out of 20000 FDA-approved explored compounds exist for LPG and  $\gamma$ -GCS drug targets. Although, *in vitro* and *in vivo* confirmations are also an inevitable requisite for further assessment of the potentiality of the predicted ligands. With the help of *in-silico* approaches identifying the molecular mechanism of action of the more active compounds was possible; thus, could define the most likely molecular target, so that the subsequent optimization using *in vitro* and *in vivo* techniques can be undertaken.

### \* Ethics

**Ethics Committee Approval:** Ethics committee approval name and number are respectively IR.AJUMS.REC and 1396614.

**Informed Consent:** A consent form was completed by all participants.

**Peer-review:** Internally peer-reviewed.

### \* Authorship Contributions

**Concept:** F.R., F.S., J.S., R.A., Design: F.R., F.S., J.S., R.A., Data Collection or Processing: F.R., F.S., J.S., R.A., Analysis or Interpretation: F.R., F.S., J.S., R.A., Literature Search: F.R., F.S., J.S., R.A., Writing: F.R., F.S., J.S., R.A.

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