ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Transcriptomics-Guided In Silico Drug Repurposing: Identifying New Candidates with Dual-Stage Antiplasmodial Activity

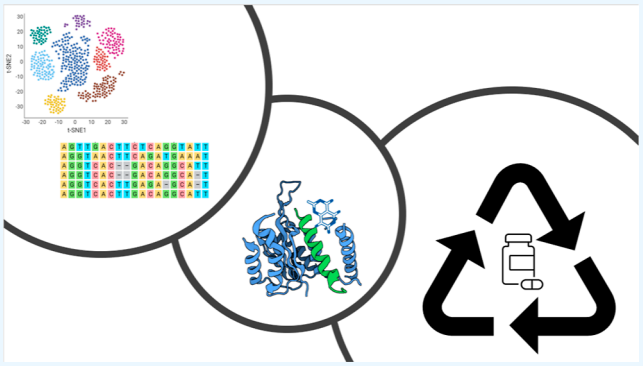
Joyce V. B. Borba, Beatriz Rosa de Azevedo, Larissa A. Ferreira, Aline Rimoldi, Luís C. Salazar Alvarez, Juliana Calit, Daniel Y. Bargieri, Fabio T. M. Costa, and Carolina Horta Andrade*

Cite This: *ACS Omega* 2023, 8, 34084−34090

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: In tropical and subtropical areas, malaria stands as a profound public health challenge, causing an estimated 247 million cases worldwide annually. Given the absence of a viable vaccine, the timely and effective treatment of malaria remains a critical priority. However, the growing resistance of parasites to currently utilized drugs underscores the critical need for the identification of new antimalarial therapies. Here, we aimed to identify potential new drug candidates against *Plasmodium falciparum*, the main causative agent of malaria, by analyzing the transcriptomes of different life stages of the parasite and identifying highly expressed genes. We searched for genes that were expressed in all stages of the parasite's life cycle, including the asexual blood stage, gametocyte stage, liver stage, and sexual stages in the insect vector, using transcriptomics data from publicly available databases. From this analysis, we found 674 overlapping genes, including 409 essential ones. By searching through drug target databases, we discovered 70 potential drug targets and 75 associated bioactive compounds. We sought to expand this analysis to similar compounds to known drugs. So, we found a list of 1557 similar compounds, which we predicted as actives and inactives using previously developed machine learning models against five life stages of *Plasmodium* spp. From this analysis, two compounds were selected, and the reactions were experimentally evaluated. The compounds HSP-990 and silvestrol aglycone showed potent inhibitory activity at nanomolar concentrations against the *P. falciparum* 3D7 strain asexual blood stage. Moreover, silvestrol aglycone exhibited low cytotoxicity in mammalian cells, transmission-blocking potential, and inhibitory activity comparable to those of established antimalarials. These findings warrant further investigation of silvestrol aglycone as a potential dual-acting antimalarial and transmission-blocking candidate for malaria control.



1. INTRODUCTION

Malaria is a parasitic disease caused by protozoa of the genus *Plasmodium* and transmitted to humans through the bite of female *Anopheles* mosquitoes. Human malaria is caused by various *Plasmodium* species, with five of them recognized as the primary etiological agents: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi*. Among these, *P. falciparum* and *P. vivax* account for the majority of malaria cases.

This disease represents a significant public health concern in many tropical and subtropical regions across the globe. Malaria is a major public health concern worldwide, with over 247 million cases and around 619,000 deaths estimated in 2021, mostly in sub-Saharan Africa.[1] The life cycle of the malaria parasite involves two hosts, namely, humans and mosquitoes. During an infected mosquito bite, sporozoites are introduced into the human host skin,[2] after which they migrate to the liver cells, undergo multiplication, and mature into schizonts. These schizonts eventually burst, releasing merozoites into the bloodstream, which invade red blood cells and continue to replicate. Eventually, the infected red blood cells rupture, freeing additional parasites from entering the bloodstream to invade new red blood cells. During this blood cycle, some parasites differentiate into sexual-stage gametocytes. The gametocytes are then ingested by *Anopheles* mosquitoes, in whose gut they form male and female gametes that fertilize to form zygotes. The zygotes mature into ookinetes and oocysts, which rupture, releasing sporozoites that migrate through the mosquito's hemolymph to invade the salivary glands. These sporozoites are then capable of infecting other humans through mosquito bites.[3,4]

The treatment of malaria is contingent upon the causative species of the parasite, the gravity of the illness, and the age and

Received: July 17, 2023
Accepted: August 18, 2023
Published: September 5, 2023

general well-being of the patient. Antimalarial medications, such as chloroquine-, mefloquine-, quinine-, and artemisinin-based combination therapies (ACTs), are frequently utilized to combat the disease. However, the emergence of resistance to both past and current antimalarial drugs emphasizes the necessity of ongoing research to remain ahead of the game. It is essential to develop new drugs, especially those with unique mechanisms of action.[5]

Drug discovery represents a complex and intricate process characterized by multiple sequential phases. It necessitates substantial investments in terms of time, resources, and collaborative efforts from interdisciplinary teams comprising scientists, clinicians, and regulatory authorities.[6] Drug repurposing refers to the strategy of identifying alternative therapeutic applications for existing drugs. Rather than developing new compounds from scratch, researchers explore the potential of approved drugs for one indication to treat other diseases.[7]

Chemogenomics combines the principles and techniques of genomics, proteomics, and chemistry to study the interactions between small molecules (such as drugs or chemical compounds) and biological systems.[8] By integrating chemical and biological data, chemogenomics enables the identification of new drug targets, the discovery of lead compounds, and the optimization of drug candidates. Therefore, combining chemogenomics with drug repurposing can be a powerful tool to identify potential drug-disease associations and repurpose approved drugs for new indications.[9,10] Furthermore, the use of computational methods, such as data mining and machine learning, can facilitate the identification of potential drug-disease pairs. By integrating and analyzing gene expression data, pathway analysis, and various data sets, computational approaches offer valuable tools for exploring novel therapeutic opportunities through drug repurposing.[11]

By utilizing therapeutic targets identified from the transcriptome of *P. falciparum*, the main goal of this work was to repurpose existing drugs as potential new candidates for antimalarial drug discovery. To achieve this, we have developed and applied a novel bioinformatics workflow that can boost the application of in silico repurposing for other diseases as well.

2. RESULTS AND DISCUSSION

The main workflow of this work is summarized in Figure 1.

2.1. Multistage Transcript *Plasmodium* Targets. Initially, an exhaustive search was conducted to identify genes exhibiting high expression levels (ranging from 80 to 100% expression) across all developmental stages of *P. falciparum*, as illustrated in Figure 2. This particular species was chosen due to the availability of extensive data encompassing most of the *Plasmodium* life stages. For the blood stage, three distinct transcriptomes were utilized: the López-Barragán transcriptome[12] (comprising ring, early trophozoites, and schizont transcriptomes), which featured 1921 highly expressed genes during the blood stage; the Bunnik transcriptome[13] (including ring, trophozoites, and schizont transcriptomes), which encompassed 1840 highly expressed genes during the blood stage; and the Zanghi transcriptome[14] (containing the ring transcriptome), which contained 1119 genes. Regarding the gametocyte stage, the López-Barragán transcriptome[12] (comprising gametocyte II/V transcriptomes) containing 1461 genes highly expressed in gametocyte stage and the Lasonder transcriptome[15] (encompassing male and female gametocyte transcriptomes) featuring 1699 highly expressed genes were selected. Given the absence of published transcriptomic data

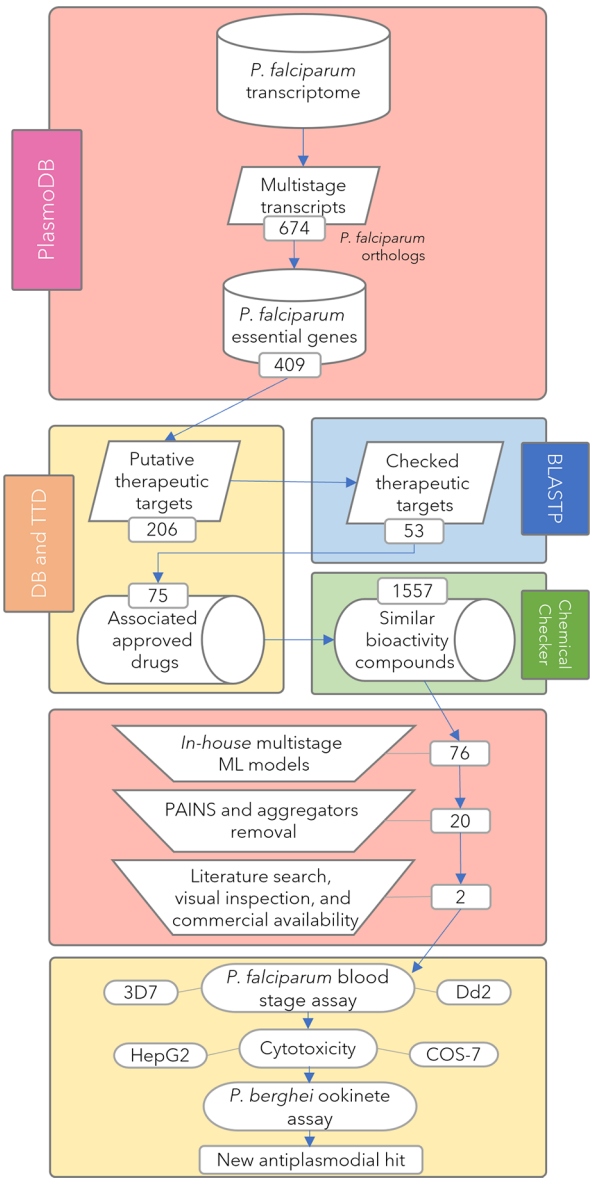


Figure 1. General chemogenomics drug repurposing pipeline used for prioritizing targets and selecting drugs and similar compounds against *P. falciparum*. DB = DrugBank; TTD = Therapeutic Target Database; ML = Machine Learning; PAINS = Pan Assay Interference compounds; 3D7 = *P. falciparum* 3D7 strain; Dd2 = *P. falciparum* Dd2 strain; HepG2 = human hepatoma cell line; and COS-7 monkey kidney fibroblast cell line.

pertaining to the liver stage of *P. falciparum*, the Cubi transcriptome,[16] encompassing the hypnozoite and liver transcriptomes of *Plasmodium cynomolgi*, served as a valuable resource. This transcriptome provided a basis for the identification of 2012 genes exhibiting significant expression levels, and subsequently, we found 1991 orthologous genes in *P. falciparum*. In the case of sexual stages, the López-Barragán[12] (containing ookinete transcriptomes) and the Zanghi[14] (including sporozoite and oocyst transcriptomes) yielded a total of 1748 highly expressed genes. Ultimately, through this meticulous process, a comprehensive set of 674 genes exhibiting overlapping expression across multiple stages was identified, with 409 of these genes being essential for the survival of the organism, according to PlasmoDB data (Table S1).

https://doi.org/10.1021/acsomega.3c05138
ACS Omega 2023, 8, 34084−34090

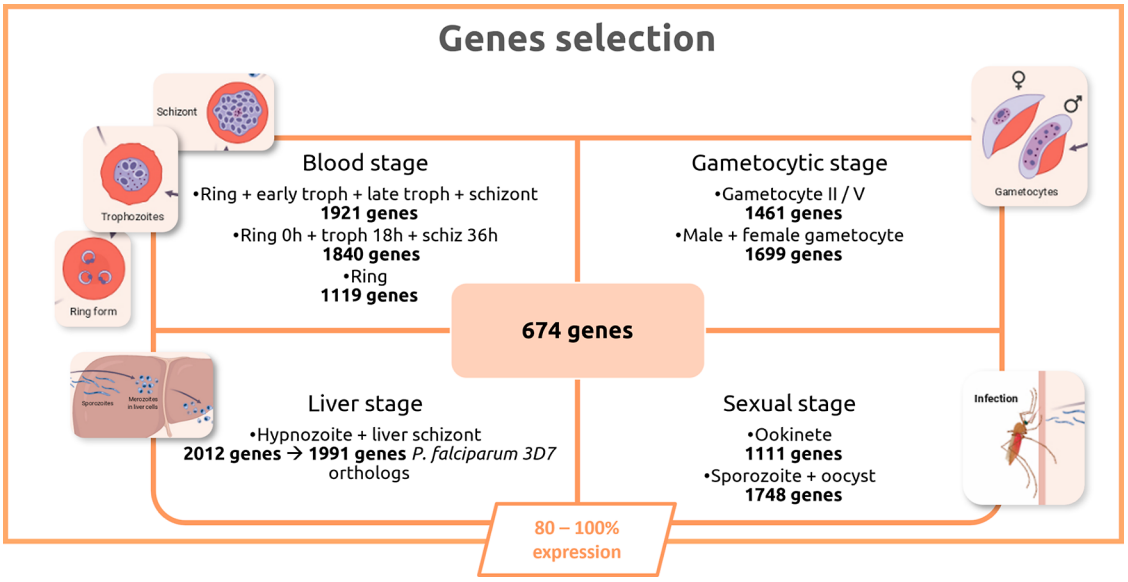


Figure 2. Overlap of highly expended genes in transcriptomes of different life stages of *P. falciparum*.

2.2. Drugs and Experimental Compounds against Multistage Targets Available in Online Databases. The *P. falciparum* genes were first individually searched in the Therapeutic Target Database (TTD).[17] There were 300 bioactive compounds associated with 147 of the 409 genes that were identified as homologous to known targets. Compounds that were removed from the market were ignored. The 409 genes were then searched in the DrugBank database (https://go.drugbank.com/), where 59 homologous targets were found. A total of 80 bioactive compounds, consisting exclusively of drugs, experimental compounds, and compounds evaluated in clinical trials, are linked to these targets in the investigation. We then combined these lists with 206 homologous targets and 378 associated compounds found.

A large number of initially discovered homologous targets had high *e*-values and thus low similarity to the sought target. These constitute a sizable proportion of those found in the TTD. The BLASTp tool was used to search for homologous targets found in TTD and DrugBank, and 70 targets with homology (*e*-value less than 1.10−30 and identity greater than 30%) were kept. Among those, 17 targets were not linked to bioactive compounds and were removed. The 53 targets kept were linked to 75 bioactive compounds in total (Table S2). Although the number of targets and composite compounds had decreased significantly, filters were required to ensure that there was minimal similarity between these targets.

2.3. Similar Compounds Found Using Chemical Checker. Based on the 75 bioactive compounds discovered in previous steps, individual searches for each drug with similar activity profiles were conducted using the Chemical Checker tool.[18] This tool allows researchers to search for similar compounds by using a bioactivity profile associated with each compound, which has five levels of complexity: chemical structure, targets, interaction networks, cellular data, and clinical data, each with five sublevels of parameters. The database contains information on approximately 800,000 compounds, the majority of which are for human pharmacological use.[18] The searches considered results with similarity only at levels higher than chemical similarity (A), target similarity (B), interaction networks (C), cellular data (D), and clinical data (E) (Figure 3).

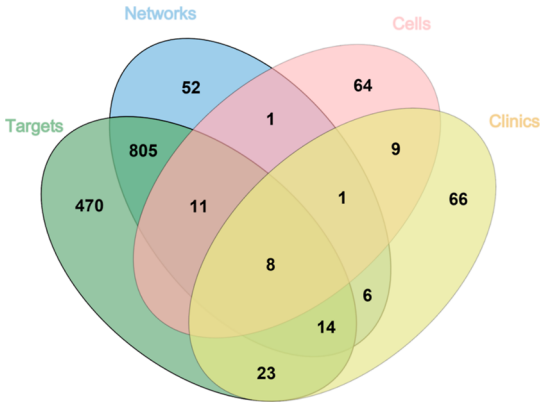


Figure 3. Venn diagram showing the 1557 similar compounds in different bioactivity profiles found using the Chemical Checker database.[18]

As we can see from Figure 3, there were 1887 compounds with some level of similarity, with 1574 (83.4%) showing similarity at the level of targets and 969 (51.3%) at the level of interaction networks but only 139 (7.3%) at the level of cellular data and 234 (12.4%) at the level of clinical data. After removing duplicates, the set of compounds contained 75 known bioactives and 1557 similar ones, for a total of 1632 compounds with potential bioactivity.

2.4. Compounds with ML Predicted Multistage Activity against *Plasmodium*. Quantitative structure−activity relationship (QSAR) models were used to screen drugs associated with therapeutic targets as well as similar compounds. The models used in this study were previously developed by the LabMol research group using machine learning algorithms. These models can predict if untested compounds will be active or inactive to inhibit the growth of *P. falciparum* sensitive (3D7) and resistant (W2) strains in the asexual stage, as well as the ookinete, gametocyte, and liver stages.

First, the 75 drugs linked to therapeutic targets expressed at various stages were assessed. Then, 23 compounds were predicted to be active against sensitive strains (3D7), 11 of

https://doi.org/10.1021/acsomega.3c05138
ACS Omega 2023, 8, 34084−34090

which were predicted to be active against resistant strains (W2), 9 of which were predicted to be active against ookinetes, 9 against the hepatic stage, and 8 against gametocytes. As a result, only eight of the 75 initial drugs were predicted to be active against the two strains and four stages of *P. falciparum* covered by the QSAR models. Artenimol, or dihydroartemisinin, a drug already in the clinic as an antimalarial drug, is one of these compounds.

The 1557 compounds similar to known drugs were then evaluated using the ML models. 529 compounds were predicted to be active against 3D7 (sensitive) strains, 94 of which were predicted to be active against resistant strains (W2), 78 of which were predicted to be active against ookinetes, 78 against liver stage, and 76 against gametocytes. As a result, only 76 of the 1557 starting compounds were predicted to be active against the two strains and four stages of *P. falciparum* covered by the models (Table S3). These 76 compounds were then checked for the presence of aggregators, and only 20 compounds passed this filter. After a visual inspection and search for commercial availability due to budget constraints, two compounds were bought for experimental evaluation: NVP_HSP990 and silvestrol aglycone (Figure 4).

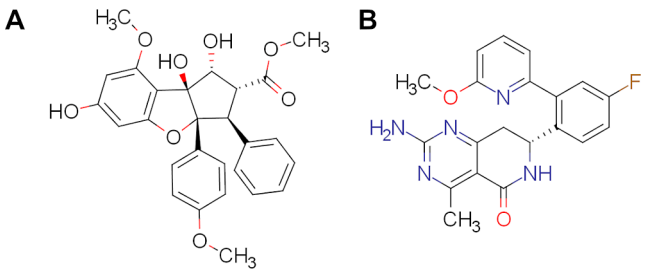


Figure 4. 2D-structure representation of (A) silvestrol aglycone and (B) NVP_HSP990.

2.5. Experimental Evaluation. An experimental evaluation was conducted to assess the efficacy of the selected compounds against *P. falciparum* 3D7 strains (chloroquine-sensitive) in order to identify potential antimalarial candidates. Moreover, we also tested our compounds for a multidrug-resistant *P. falciparum* strain, Dd2, which is known for its resistance to a broader range of antimalarial drugs compared to W2. Phenotypic screening was performed initially at a concentration of 5 μM with the 3D7 strain, revealing that both compounds exhibited more than 50% inhibition of parasite growth. Further evaluation was conducted to determine the EC_{50} values of the compounds in both parasite strains, as depicted in Figure 5 and Table 1. Notably, both compounds were found to inhibit parasite growth at nanomolar concentrations in both strains.

Of particular interest, silvestrol aglycone demonstrated exceptional efficacy in inhibiting parasite growth, exhibiting a similar EC_{50} value as that of the established antimalarials. In fact, the inhibition curve of silvestrol aglycone closely resembled those of chloroquine and artesunate in the same strain (Figure S1). Additionally, the compounds were tested for their cytotoxicity, and silvestrol aglycone exhibited a selectivity index of 34, further confirming its potential as a repurposed antimalarial compound.

Given the promising results of silvestrol aglycone, further investigations were conducted to assess its transmission-blocking potential using the ookinete conversion inhibition assay.[19] The results showed that silvestrol aglycone was able to inhibit 99.65% of ookinete conversion at 10 μM, compared to the untreated control samples. Furthermore, a dose−response curve (Figure S2) was constructed, revealing an EC_{50} value of 3.21 μM for the inhibition of ookinete conversion in *Plasmodium berghei*, supporting the efficacy of silvestrol aglycone as an antimalarial compound with transmission-blocking potential

3. CONCLUSIONS

By combining the power of chemogenomics with drug repurposing, we harnessed a potent tool to identify potential drug-disease associations and repurpose approved drugs for *P. falciparum*. We have analyzed highly expressed genes across all stages of the parasite's life cycle, identifying homologous targets and associated compounds. This integration allowed us to leverage the vast knowledge and data available on approved drugs, expanding the scope of our investigation beyond traditional drug discovery methods. By leveraging the Chemical Checker tool, we identified similar compounds and their potential bioactivity, enhancing our understanding of the existing drug/compound landscape. Consequently, this comprehensive approach led to the identification of 1557 potential bioactive compounds. Through the implementation of QSAR models, we further refined our selection to 75 compounds linked to therapeutic targets and predicted to be active against both sensitive and resistant strains of *P. falciparum* across all stages of its life cycle. Among these compounds, NVP-HSP990, and silvestrol aglycone demonstrated remarkable inhibitory activity at nanomolar concentrations against the asexual blood stage. Notably, silvestrol aglycone exhibited potent inhibition comparable to the established antimalarials, along with low

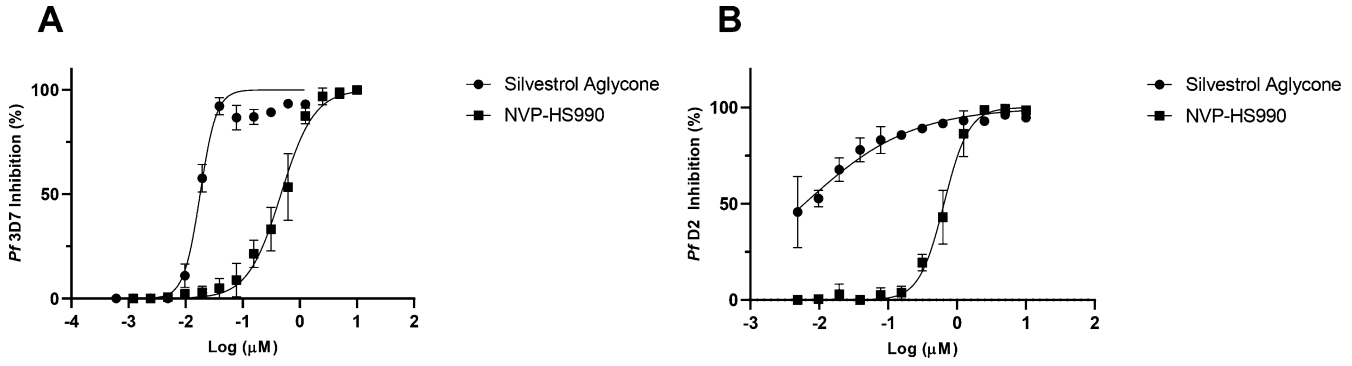


Figure 5. In vitro antimalarial activity of selected compounds against *P. falciparum*. (A) Inhibition curves of silvestrol aglycone and NVP-HSP990 against chloroquine sensitive (3d7) and (B) multidrug-resistant (Dd2) *P. falciparum* strains.

https://doi.org/10.1021/acsomega.3c05138
ACS Omega 2023, 8, 34084−34090

Table 1. EC_{50} of Compounds Tested against Chloroquine-Sensitive (3D7), Multidrug-Resistant (Dd2) *P. falciparum* Strains, Cytotoxicity (CC_{50}) on Mammalian Cell Lines (COS-7 and HepG2), and Percent of Inhibition of Ookinete Formation (Transmission Blocking Activity)

	EC_{50} (μM)		CC_{50} (μM)				
compound	*Pf*3D7[a]	*Pf*Dd2[b]	COS-7[c]	HepG2[d]	SI[e]	SI[f]	transmission blocking (%)
silvestrol aglycone	0.0094 ± 0.0007	0.006 ± 0.003	0.32 ± 0.2	0.24 ± 0.2	34	25	99.65
NVP-HSP990	0.54 ± 0.05	0.66 ± 0.1	0.09 ± 0.1	2.69 ± 3.7	0.2	4.9	57.35
chloroquine	0.0035 ± 0.0004	0.056 ± 0.01					

[a]Half maximal effective concentration in 3D7 strain. [b]Half maximal effective concentration in Dd2 strain. [c]Half maximal cytotoxic concentration on COS-7 cell. [d]Half maximal cytotoxic concentration on HEPG2 cell. [e]Selectivity index calculated between CC_{50} COS-7 and EC_{50} 3D7 strains. [f]Selectivity index calculated between CC_{50} HEPG2 and EC_{50} 3D7 strains.

cytotoxicity and promising transmission-blocking properties. These findings highlight the potential of silvestrol aglycone as a dual-acting candidate for the development of new antimalarial drugs.

4. MATERIALS AND METHODS

4.1. Multistage (Transcript) Targets Search in Online Databases. The first step in this work was searching for the malarial transcriptome, which is available at the PlasmoDB online platform.[20] We prioritized transcripts identified in *P. falciparum* and highly expressed (≥80% expression) simultaneously in all life stages. In addition, we compared those with the organism's essential genes[21] and selected the multistage transcripts identified as essential for further usage.

4.2. Active Drugs and Experimental Compounds against Multistage Targets in Online Databases. The predicted amino acid sequences of the multistage targets were then used in a sequence similarity search for known therapeutic targets. Two databases were consulted: DrugBank (https://go.drugbank.com) and Therapeutic Target Database (TTD, dp.idrblab.net/ttd), which provided similar therapeutic target genes and associated drugs to each sequence consulted. Moreover, the similarity between the multistage target sequences and the therapeutic target genes found was checked using BLASTp.[22]

4.3. Similar Compounds Search by Bioactivity Profile Using Chemical Checker. The drug set found to be associated to therapeutic target genes was explored to find new compounds with possible antimalarial applications, using the online tool Chemical Checker.[18] This tool compares any chemical compound to a database of more than 800,000 compounds using five levels of complexity on the bioactivity profile. Each drug was searched, and all compounds with similar bioactivity profile were added to an expanded compounds set.

4.4. Virtual Screening Using Machine Learning Models. The sets of drugs and compounds selected from online databases were virtually screened to identify potential candidates for repurposing against *P. falciparum*. To accomplish this, a set of *in-house* QSAR models developed with machine learning (QSAR-ML) algorithms were used. These models were designed to predict the activity of compounds at different stages of the *Plasmodium* life cycle, including the blood stage (3D7 and W2 strains), liver stage, gametocyte stage, and ookinete stage. The models were generated using the random forest (RF) algorithm, a widely used the ML technique. To extract and interpret the structural and chemical information from the databases and inserted samples, the models utilized various molecular descriptors, such as ECFP descriptors for the 3D7 and W2 models, molecular access system (MACCS) descriptors for the ookinete model, hybrid Mordred and ECFP descriptors for the gametocyte model, and FCFP descriptors for the liver model. Compounds that were predicted to exhibit activity by all five models underwent a filtering process and were considered as potential candidates for experimental evaluation.

4.5. *P. falciparum* In Vitro Culture. Phenotypic assays were performed using in vitro strains of sensitive *P. falciparum* (3D7) and multidrug-resistant strains (Dd2).[23] Briefly, *P. falciparum* was cultured in erythrocytes of type O+ humans with a final hematocrit of 5% in complete medium (RPMI-1640 containing 10% of A+ human plasma and 0.05 mg/mL gentamycin at pH 7.4) maintained at 37 °C with 5% CO_2, 5% O_2, and 90% N_2, as previously described by Trager and Jensen.[24] Parasitemia was monitored daily using smears stained with Giemsa and visualized under an optical microscope. The synchronization of the ring stage culture was performed with a 5% D-sorbitol solution before the experiments.

4.6. In Vitro Assays for *P. falciparum* Inhibition. Inhibitory concentration assays of compounds in *P. falciparum* strains were performed in 96-well culture plates with 0.5% parasitemia and 2% hematocrit in lines 3D7 and Dd2. All assays presented a positive control (untreated wells) and a negative control (wells treated with 5 μM of artesunate). In addition, chloroquine was used as a standard antimalarial in the EC_{50} assays. The plates were incubated at 37 °C under an atmosphere of 5% CO_2, 5% O_2, and 90% N_2 for 72 h. At the end of the incubation, the plates were frozen for 24 h, subsequently thawed, and parasite suspensions were revealed using the SYBR Green protocol (Sigma).[25] The fluorescence reading was performed in a CLARIOstar plate reader at the wavelengths 483 nm excitation−530 nm emission. The growth inhibition values were expressed as percentages relative to the drug-free control, and EC_{50} values were calculated by plotting log dosing vs growth inhibition (expressed as percentage relative to the drug-free control) using GraphPad Prism 8. The experiments were carried out in three independent assays.

4.7. In Vitro Assay for Ookinete Conversion Inhibition. To determine the compounds activity against the sexual stages of *P. berghei*, we used the murine model PbOokluc, described by Calit and co-workers.[19] First, the compounds were diluted in an ookinete medium[26] to a final concentration of 10 μM and a final volume of 40 μL. Afterward, 4 μL of mouse blood infected with the PbOokluc line containing gametocytes was added and incubated at 21 °C for 24 h. After 24 h the nanoluciferase activity was measured using a plate luminometer. In addition, the EC_{50} was determined using a similar experiment, applying a 2-fold serial dilution of the compound and decreasing it until no inhibition was observed. The EC_{50} value and curve were calculated using the program GraphPad Prism 8.0.

4.8. Cytotoxicity Assays. The cytotoxicity of the compounds was tested using MTT assays (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide]) to measure the

https://doi.org/10.1021/acsomega.3c05138
ACS Omega 2023, 8, 34084−34090

metabolic reduction of bromide on mitochondrial enzymes in two mammalian cell lines: Hep-G2 cells (human hepatoma) and COS-7 cells (monkey kidney fibroblasts). The cells were grown in DMEM medium supplemented with 10% inactivated fetal bovine serum and incubated at 5% CO_2 and 37 °C. Assays were performed in 96-well microplates at a density of 10^4 cells per well in a serial dilution of the drugs (100−0.032 μM) and untreated cells (Control). The microplates were then incubated for 72 h. After 72 h, 15 μL of MTT solution (5 mg/mL) was added. After 4 h of incubation, the plates were centrifuged at 1500 rpm for 5 min; the pellet was washed with PBS and resuspended in 50 μL of isopropanol. The amount of formazan produced is proportional to the number of living cells. The absorbance reading (A570) was performed on a CLARIOstar plate reader. CC_{50} values were calculated by plotting a log dose vs the viability curve in GraphPad Prism 8, and the selectivity index was calculated between CC_{50} HEPG2 or COS-7 and EC_{50} 3D7 strain. The experiments were carried out in three independent assays.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c05138.

In vitro antimalarial inhibition curves of silvestrol aglycone, NVP_HSP990, artesunate, and chloroquine against *P. falciparum* sensitive (3d7) and resistant (W2) strains and ookinete inhibition curve of silvestrol aglycone in three independent experiments (PDF)

Genes essential for survival, highly expressed, and overlapping across all *Plasmodium* stages; prioritized targets linked to their bioactive ligands; and compounds predicted to be active against four stages of malaria (XLSX)

■ AUTHOR INFORMATION

Corresponding Author

Carolina Horta Andrade − *Laboratory for Molecular Modeling and Drug Design (LabMol), Faculdade de Farmacia, Universidade Federal de Goias, 74605-170 Goiânia, Goiás, Brazil;* orcid.org/0000-0003-0101-1492; Email: carolina@ufg.br

Authors

Joyce V. B. Borba − *Laboratory for Molecular Modeling and Drug Design (LabMol), Faculdade de Farmacia, Universidade Federal de Goias, 74605-170 Goiânia, Goiás, Brazil; Laboratory of Tropical Diseases—Prof. Dr. Luiz Jacintho da Silva, Department of Genetics Evolution, Microbiology and Immunology, University of Campinas, 13083-970 Campinas, São Paulo, Brazil;* orcid.org/0000-0002-2663-5173

Beatriz Rosa de Azevedo − *Laboratory for Molecular Modeling and Drug Design (LabMol), Faculdade de Farmacia, Universidade Federal de Goias, 74605-170 Goiânia, Goiás, Brazil*

Larissa A. Ferreira − *Laboratory of Tropical Diseases—Prof. Dr. Luiz Jacintho da Silva, Department of Genetics Evolution, Microbiology and Immunology, University of Campinas, 13083-970 Campinas, São Paulo, Brazil*

Aline Rimoldi − *Laboratory of Tropical Diseases—Prof. Dr. Luiz Jacintho da Silva, Department of Genetics Evolution, Microbiology and Immunology, University of Campinas, 13083-970 Campinas, São Paulo, Brazil*

Luís C. Salazar Alvarez − *Laboratory of Tropical Diseases—Prof. Dr. Luiz Jacintho da Silva, Department of Genetics Evolution, Microbiology and Immunology, University of Campinas, 13083-970 Campinas, São Paulo, Brazil*

Juliana Calit − *Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo, 05508-000 São Paulo, São Paulo, Brazil*

Daniel Y. Bargieri − *Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo, 05508-000 São Paulo, São Paulo, Brazil*

Fabio T. M. Costa − *Laboratory of Tropical Diseases—Prof. Dr. Luiz Jacintho da Silva, Department of Genetics Evolution, Microbiology and Immunology, University of Campinas, 13083-970 Campinas, São Paulo, Brazil*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c05138

Funding

The authors would like to thank Brazilian funding agencies CNPq (441038/2020-4), CAPES-STINT (88881.304811/2018-01), FAPEG (202010267000272), FAPESP (Grants 2017/18611-7 and 2021/06769-0), and to the Swedish Research Council (grants 2016-05627 and 2021-03667). J.V.B.B. and J.C. were funded by FAPESP 2019/21854-4 and 2018/24878-9, respectively. L.C.S.A. was funded by CNPq 162117/2018-3. DYB was funded by FAPESP grant 2021/06769-0 and Instituto Serrapilheira grant G-1709-16618. C.H.A. and F.T.M.C. are CNPq research fellows.

Notes

The authors declare no competing financial interest.

■ DEDICATION

△J.V.B.B. and B.R.d.A. contributed equally to this work.

■ REFERENCES

(1) World Health Organization. *World Malaria Report 2022*; World Health Organization, 2022.

(2) Ménard, R.; Tavares, J.; Cockburn, I.; Markus, M.; Zavala, F.; Amino, R. Looking under the Skin: The First Steps in Malarial Infection and Immunity. *Nat. Rev. Microbiol.* **2013**, *11* (10), 701−712.

(3) Phillips, M. A.; Burrows, J. N.; Manyando, C.; van Huijsduijnen, R. H.; Van Voorhis, W. C.; Wells, T. N. C. Malaria. *Nat. Rev. Dis. Primers* **2017**, *3* (1), 17050.

(4) Tebben, K.; Bradwell, K.; Serre, D. Variation in Selective Constraints along the Plasmodium Life Cycle. *Infect., Genet. Evol.* **2021**, *92*, 104908.

(5) Tse, E. G.; Korsik, M.; Todd, M. H. The Past, Present and Future of Anti-Malarial Medicines. *Malar. J.* **2019**, *18* (1), 93.

(6) Morimoto, B. H.; Castelloe, E.; Fox, A. W. Safety Pharmacology in Drug Discovery and Development. *Handb. Exp. Pharmacol.* **2015**, *229*, 65−80.

(7) Pushpakom, S.; Iorio, F.; Eyers, P. A.; Escott, K. J.; Hopper, S.; Wells, A.; Doig, A.; Guilliams, T.; Latimer, J.; McNamee, C.; et al. Drug Repurposing: Progress, Challenges and Recommendations. *Nat. Rev. Drug Discovery* **2019**, *18* (1), 41−58.

(8) Andrade, C. H.; Neves, B. J.; Melo-Filho, C. C.; Rodrigues, J.; Silva, D. C.; Braga, R. C.; Cravo, P. V. L. In Silico Chemogenomics Drug Repositioning Strategies for Neglected Tropical Diseases. *Curr. Med. Chem.* **2019**, *26*, 4355−4379.

(9) Jones, L. H.; Bunnage, M. E. Applications of Chemogenomic Library Screening in Drug Discovery. *Nat. Rev. Drug Discovery* **2017**, *16* (4), 285−296.

(10) Kubinyi, H. Chemogenomics in Drug Discovery. *Chem. Biol.* **2006**, *58*, 1−19.

(11) Sabe, V. T.; Ntombela, T.; Jhamba, L. A.; Maguire, G. E. M.; Govender, T.; Naicker, T.; Kruger, H. G. Current Trends in Computer Aided Drug Design and a Highlight of Drugs Discovered via Computational Techniques: A Review. *Eur. J. Med. Chem.* **2021**, *224*, 113705.
(12) López-Barragán, M. J.; Lemieux, J.; Quiñones, M.; Williamson, K. C.; Molina-Cruz, A.; Cui, K.; Barillas-Mury, C.; Zhao, K.; Su, X. Directional Gene Expression and Antisense Transcripts in Sexual and Asexual Stages of Plasmodium Falciparum. *BMC Genomics* **2011**, *12* (1), 587.
(13) Bunnik, E. M.; Chung, D.-W.; Hamilton, M.; Ponts, N.; Saraf, A.; Prudhomme, J.; Florens, L.; Le Roch, K. G. Polysome Profiling Reveals Translational Control of Gene Expression in the Human Malaria Parasite Plasmodium Falciparum. *Genome Biol.* **2013**, *14* (11), R128.
(14) Zanghì, G.; Vembar, S. S.; Baumgarten, S.; Ding, S.; Guizetti, J.; Bryant, J. M.; Mattei, D.; Jensen, A. T. R.; Rénia, L.; Goh, Y. S.; et al. A Specific PfEMP1 Is Expressed in P. Falciparum Sporozoites and Plays a Role in Hepatocyte Infection. *Cell Rep.* **2018**, *22* (11), 2951−2963.
(15) Lasonder, E.; Rijpma, S. R.; van Schaijk, B.; Hoeijmakers, W. A. M.; Kensche, P. R.; Gresnigt, M. S.; Italiaander, A.; Vos, M. W.; Woestenenk, R.; Bousema, T.; et al. Integrated Transcriptomic and Proteomic Analyses of P. Falciparum Gametocytes: Molecular Insight into Sex-Specific Processes and Translational Repression. *Nucleic Acids Res.* **2016**, *44* (13), 6087−6101.
(16) Cubi, R.; Vembar, S. S.; Biton, A.; Franetich, J.-F.; Bordessoulles, M.; Sossau, D.; Zanghi, G.; Bosson-Vanga, H.; Benard, M.; Moreno, A.; et al. Laser Capture Microdissection Enables Transcriptomic Analysis of Dividing and Quiescent Liver Stages of Plasmodium Relapsing Species. *Cell. Microbiol.* **2017**, *19* (8), No. e12735.
(17) Zhou, Y.; Zhang, Y.; Lian, X.; Li, F.; Wang, C.; Zhu, F.; Qiu, Y.; Chen, Y. Therapeutic Target Database Update 2022: Facilitating Drug Discovery with Enriched Comparative Data of Targeted Agents. *Nucleic Acids Res.* **2022**, *50* (D1), D1398−D1407.
(18) Duran-Frigola, M.; Pauls, E.; Guitart-Pla, O.; Bertoni, M.; Alcalde, V.; Amat, D.; Juan-Blanco, T.; Aloy, P. Extending the Small-Molecule Similarity Principle to All Levels of Biology with the Chemical Checker. *Nat. Biotechnol.* **2020**, *38*, 1087−1096.
(19) Calit, J.; Dobrescu, I.; Gaitán, X. A.; Borges, M. H.; Ramos, M. S.; Eastman, R. T.; Bargieri, D. Y. Screening the Pathogen Box for Molecules Active against Plasmodium Sexual Stages Using a New Nanoluciferase-Based Transgenic Line of P. Berghei Identifies Transmission-Blocking Compounds. *Antimicrob. Agents Chemother.* **2018**, *62*, e01053-18,
(20) Aurrecoechea, C.; Brestelli, J.; Brunk, B. P.; Dommer, J.; Fischer, S.; Gajria, B.; Gao, X.; Gingle, A.; Grant, G.; Harb, O. S.; et al. PlasmoDB: A Functional Genomic Database for Malaria Parasites. *Nucleic Acids Res.* **2009**, *37* (Database), D539−D543.
(21) Zhang, M.; Wang, C.; Otto, T. D.; Oberstaller, J.; Liao, X.; Adapa, S. R.; Udenze, K.; Bronner, I. F.; Casandra, D.; Mayho, M.; et al. Uncovering the Essential Genes of the Human Malaria Parasite Plasmodium Falciparum by Saturation Mutagenesis. *Science* **2018**, *360* (6388), No. eaap7847.
(22) Johnson, M.; Zaretskaya, I.; Raytselis, Y.; Merezhuk, Y.; McGinnis, S.; Madden, T. L. NCBI BLAST: A Better Web Interface. *Nucleic Acids Res.* **2008**, *36* (Web Server), W5−W9.
(23) Wellems, T. E.; Plowe, C. V. Chloroquine-Resistant Malaria. *J. Infect. Dis.* **2001**, *184* (6), 770−776.
(24) Trager, W.; Jensen, J. Human Malaria Parasites in Continuous Culture. *Science* **1976**, *193* (4254), 673−675.
(25) Hartwig, C. L.; Ahmed, A. O. A.; Cooper, R. A.; Stedman, T. T. SYBR Green I-Based Parasite Growth Inhibition Assay for Measurement of Antimalarial Drug Susceptibility in Plasmodium Falciparum. In *Methods in Malaria Research*; Moll, K., Kaneko, A., Scherf, A., Wahlgren, M., Eds.; EVIMalaR: Manassas, 2013; pp 122−129.
(26) Blagborough, A. M.; Delves, M. J.; Ramakrishnan, C.; Lal, K.; Butcher, G.; Sinden, R. E. Assessing Transmission Blockade in Plasmodium Spp. In *Methods in Molecular Biology (Methods and Protocols)*; Ménard, R., Ed.; Humana Press: Totowa, NJ, 2012; pp 577−600..

https://doi.org/10.1021/acsomega.3c05138
ACS Omega 2023, 8, 34084−34090