RESEARCH ARTICLE

In silico screening of chloroquine analogues for compounds with more affinity for the *Plasmodium falciparum* chloroquine transporter as potential antimalarial drugs [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

Background: Malaria is an acute febrile illness affecting over 229 million people worldwide. Children aged five years and below are affected the most, with the highest prevalence in Sub-Saharan Africa. Chloroquine was previously used as the first-line treatment for malaria due to its affordability and high efficacy, but resistance has developed. Resistance to chloroquine is due to a mutation in the protein *Plasmodium falciparum* Chloroquine Transporter (pfcRT) which effluxes the drug from the parasitic digestive vacuole, decreasing the drug concentration. Resistance has however been shown to be reversible by compounds that can bind to the protein.

Methods: *In silico* screening for chloroquine analogues was done using SwissSimilarity, SWISSADME, SwissTargetPrediction, Pubchem sketcher, Chimera and Avogadro tools to predict pharmacodynamics and pharmacokinetic profiles of the selected analogues.

Results: About 20 compounds with a similarity index of > 95% were obtained from the ZINC database. In total, 12 of the 20 compounds showed a higher binding affinity to the mutant pfcRT protein. Overall, four of the 12 had a binding affinity less than -8.0 compared to -7.0 for chloroquine. Compound ZINC01596768 had the greatest binding strength at -8.3. The other analogues were ZINC38050614, ZINC38050617, and ZINC38050615 with binding interaction strengths of -8.0, -8.2 and -8.2 respectively. Pharmacokinetic profile prediction showed all 12 compounds inhibited the enzymes CYP1A2 and CYP2D6, followed the Lipinski rules, had a high GI absorption, were permeant to the blood brain barrier, had no alerts on the PAINS criteria and had violated the rule of XLOGP3 > 3.5 in lead likeness. Compounds ZINC38050614, ZINC38050617, and ZINC38050615 were predicted to be substrates of P-glycoprotein. The synthetic accessibility score for the twelve compounds were below 3.07.

Open Peer Review

Approval Status

	1	2
version 1
15 Feb 2022	view	view

1. David A. Fidock , Columbia University
Irving Medical Center, New York, USA

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Adama Science and Technology University
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Any reports and responses or comments on the article can be found at the end of the article.

Conclusions: Results demonstrated that the compounds ZINC01596768, ZINC38050614, ZINC38050617, and ZINC38050615 were potential candidates that could be tested and developed as co-formulations of chloroquine.

Keywords

chloroquine, chloroquine analogue, SwissSimilarity, SWISSADME, SwissTargetPrediction, Pubchem sketcher, chimera, Avogadro, pharmacokinetic, pharmacodynamics, resistance, pfCRT

This article is included in the **Chemical Information Science** gateway.

Corresponding author: Michael Walekhwa (walekhwa@gmail.com)

Author roles: **Otieno F:** Data Curation, Formal Analysis, Investigation, Methodology, Writing – Original Draft Preparation; **Walekhwa M:** Conceptualization, Methodology, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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Introduction

Malaria is an acute febrile infection caused by *Plasmodium* parasites. The parasite is transmitted through the bite of an infected female *Anopheles* mosquito when it is feeding on human blood. Five species of plasmodium parasites exist of which *Plasmodium falciparum* and *Plasmodium vivax* have been shown to cause the greatest morbidity and mortality in humans. Malaria cases in the African, South East Asian and Eastern Mediterranean region are commonly caused by the *P. falciparum* species while the *P. vivax* species has been implicated mostly with malarial cases from the American region (Ocan *et al.*, 2019).

Epidemiology

Malaria is an infection that is most prevalent in tropical regions across the world. According to the World Health Organization (WHO) report on malaria disease burden as of 2019, there were 229 million cases reported worldwide, which was an increase by one million cases from the previous year (WHO, 2021). Worldwide mortality due to malaria in 2019 was at 409,000, representing a decrease by 2,000 from the year 2018. Children less than 5 years of age across the world accounted for 67% of the total cases and deaths. Moreover, the African region represented the greatest proportion of these cases and deaths, contributing to approximately 94% of the total cases as of 2019 (Fact sheet about malaria, WHO, 2021).

Statement of problem

Previously, chloroquine was the first-line agent for treating all forms of malaria due to its affordability, usability and high efficacy but with time, the parasites *P. falciparum* and *P. vivax* developed resistance to it (Al-Bari, 2015). Currently, the first-line agent for malaria involves using artemisinin-based combination therapy (ACT). Though these drug combinations currently in use are effective, they are costly and have a high pill burden. Resistance to chloroquine by *P. falciparum* was first isolated from South East Asia and it spread across the world. Scholars suggest that resistance to chloroquine led to a doubling of deaths associated with malaria within the sub-Saharan Africa (Kim *et al.*, 2020).

Resistance to chloroquine by *P. falciparum* has been shown to be mediated by the mutant form of the protein *P. falciparum* chloroquine-resistant transporters (pfCRT) which belongs to the superfamily of transporters called the drug/metabolite transporter (DMT) superfamily (Kim *et al.*, 2020). The protein is a ten transmembrane helix spanning the membrane of the digestive vacuole of the parasite with five helical hairpins. Helices 1-4 and 6-9 form a central cavity acting as a channel for removing molecules from the vacuole (Kim *et al.*, 2020).

The mechanism of resistance by this mutant protein involves actively binding chloroquine and subsequently effluxing it from the vacuole, leading to a decrease in chloroquine concentration within the vacuole. Chloroquine-associated mutations occur in the K76T region of amino acids which is located directly in the lining of the central cavity (Ocan *et al.*, 2019). These mutations cause an increase in the affinity of chloroquine-binding, leading to increase in efflux rate. Moreover, pfCRT mutations have been shown to also induce resistance to other antimalarial drugs such as the quinolone derivatives (Sanchez *et al.*, 2019). Other studies have shown that drugs such as verapamil reduce the binding of chloroquine to the protein causing a reversal in its resistance nature (Bellanca *et al.*, 2014).

This study aimed to identify compounds with higher binding affinity to the mutant pfCRT and, in addition, a high or similar antimalarial efficacy as chloroquine, so as to reduce efflux of the parent drug (chloroquine) from the parasitic digestive vacuole while also exacting antimalarial activity. The study will focus on compounds with chloroquine similarity percentage of 95% and above.

Methods

Identification of compounds similar to chloroquine was done through *in silico* search where a ligand-based virtual screening for chloroquine analogues was carried out on the ZINC database (ZINC15). The search was done by feeding the canonical smiles of chloroquine into SwissSimilarity and running it. The search is based on finding compounds with similar pharmacophore, molecular fingerprint and shape of the parent drug.

Compounds from the search results with a similarity index of 95% were isolated and then drawn using Pubchem sketcher V2.4. Three-dimensional models of the compounds were generated through Avogadro version 1.1.0 after which they were prepared through optimization in Avogadro and minimization in Chimera version 1.14c. The 3D structure of the pfCRT protein was retrieved from the Protein Databank-101 (accession number: 2B9L <https://www.rcsb.org/>) and standardized using Chimera 1.14c. Finally, surface binding analysis of the prepared compounds was carried out by docking them with the standardized pfCRT protein. The docking scores showing the binding strength of the compounds with the protein were generated and compared with that of chloroquine.

In addition, prediction of the pharmacokinetic profile of the selected compounds was done using the web tool SWISSADME and also prediction of other likely binding sites of the drug within the body was carried out using the web tool SwissTargetPrediction.

Results

Table 1. Docking scores of selected compounds with similarity index of 95% and above showing their binding affinities in comparison to that of chloroquine.

	Zinc identifiers	Canonical smiles	Similarity index	Docking scores
	Chloroquine	<chem>CCN(CC)CCCC(C)NC1=C2C=CC(=CC2=NC=C1)Cl</chem>	1.000	-7.0
1	ZINC02042694	<chem>CCNCCCC(Nc1ccnc2c1ccc(c2)Cl)C</chem>	0.993	-6.9
2	ZINC01873617	<chem>CCNCCCC(Nc1ccnc2c1ccc(c2)Cl)C</chem>	0.993	-7.1
3	ZINC02042606	<chem>CNCCCC(Nc1ccnc2c1ccc(c2)Cl)C</chem>	0.989	-6.8
4	ZINC96331701	<chem>CN(C[C@H]1CCC[C@H]1CNc1ccnc2c1ccc(c2)Cl)C</chem>	0.988	-7.8
5	ZINC01729567	<chem>CN(CCCCNc1ccnc2c1ccc(c2)Cl)C</chem>	0.984	-6.8
6	ZINC01596768	<chem>Clc1ccc2c(c1)nc2c2NCCCNc1CCCC1</chem>	0.975	-8.3
7	ZINC06020572	<chem>NCCCC(Nc1ccnc2c1ccc(c2)Cl)C</chem>	0.974	-6.9
8	ZINC78617776	<chem>CN([C@H]1CCCN(C1)c1ccnc2c1ccc(c2)Cl)C</chem>	0.974	-7.3
9	ZINC78617773	<chem>CN(C1CCCN(C1)c1ccnc2c1ccc(c2)Cl)C</chem>	0.974	-7.7
10	ZINC06036375	<chem>NCCC[C@H](Nc1ccnc2c1ccc(c2)Cl)C</chem>	0.972	-7.0
11	ZINC01542123	<chem>CCN(CCCNc1ccnc2c1ccc(c2)Cl)CC</chem>	0.962	-7.2
12	ZINC06176159	<chem>CN(CCC(Nc1ccnc2c1ccc(c2)Cl)C)C</chem>	0.959	-7.3
13	ZINC06175381	<chem>CN(CCC(Nc1ccnc2c1ccc(c2)Cl)C)C</chem>	0.959	-7.1
14	ZINC01683221	<chem>Clc1ccc2c(c1)nc2c2NCCNc1CCCC1</chem>	0.957	-7.5
15	ZINC38050614	<chem>N[C@H]1CCCC[C@H]1Nc1ccnc2c1ccc(c2)Cl</chem>	0.956	-8.0
16	ZINC38050617	<chem>N[C@@H]1CCCC[C@H]1Nc1ccnc2c1ccc(c2)Cl</chem>	0.952	-8.2
17	ZINC19721342	<chem>NCCCCNc1ccnc2c1ccc(c2)Cl</chem>	0.951	-6.8
18	ZINC38050615	<chem>N[C@H]1CCCC[C@H]1Nc1ccnc2c1ccc(c2)Cl</chem>	0.950	-8.2

Table 2. prediction of other possible binding sites for the analogues within the body.

Zinc ID	Swiss target prediction (with probability above 19%)
ZINC01873617	HERG voltage gated channel H ₃ receptor Histamine N-methyl transferase Quinone reductase 2 M ₂ receptor Alpha ₁ receptor Norepinephrine transporter
ZINC96331701	H ₃ receptor Histamine N-methyl transferase Quinone reductase 2
ZINC01596768	H ₃ receptor Histamine N-methyl transferase
ZINC78617776	H ₃ receptor
ZINC78617773	HERG voltage gated channel
ZINC01542123	H ₃ receptor Histamine N-methyl transferase
ZINC06176159	H ₃ receptor
ZINC06175381	H ₃ receptor
ZINC01683221	5-HT _{2A}
ZINC38050614	H ₃ receptor
ZINC38050617	H ₃ receptor
ZINC38050615	H ₃ receptor

Table 3. prediction of pharmacokinetic profile of the selected analogues by SWISSADME.

Zinc IDs	Lipophilicity log $P_{(O/W)}$	P-gp substrate	Inhibition OF CYP50		Synthetic accessibility
			CYP2C19	CYP3A4	
ZINC01873617	3.57	No	Yes	Yes	2.54
ZINC96331701	3.89	No	No	Yes	3.06
ZINC01596768	4.15	No	Yes	No	2.33
ZINC78617776	3.08	No	No	No	2.59
ZINC78617773	3.08	No	No	No	2.59
ZINC01542123	3.52	No	Yes	No	2.17
ZINC06176159	3.14	No	No	No	2.49
ZINC06175381	3.14	No	No	No	2.49
ZINC01683221	3.83	No	Yes	No	2.21
ZINC38050614	3.03	Yes	Yes	No	2.71
ZINC38050617	3.02	Yes	Yes	No	2.71
ZINC38050615	3.03	Yes	Yes	No	2.71
Lipinski rule (druglikeness)	All the selected compounds obeyed Lipinski rules with zero violation				
GI absorption	All the selected compounds have a high GI absorption				
Permeates BBB	All the selected compounds permeate the blood-brain barrier				
Inhibits cyp450	All the selected compounds inhibit the enzyme CYP1A2 and CYP2D6 All the selected compounds did not inhibit the enzyme CYP2C9				
Bioavailability (BA) score	All the selected compounds had a BA of 0.55				
Alerts on PAINS criteria	All the selected compounds had zero alerts				
Leadlikeness	All the selected compounds had a single violation; XLOGP3 > 3				

Discussion

P. falciparum resistance to chloroquine is not attributed to drug modification or inactivation, but rather due to increase in efflux of the drug from the digestive vacuole of the parasite (Chinappi *et al.*, 2010; Sidhu *et al.*, 2002). This decreases the concentration of the drug within the vacuole such that it becomes ineffective in completely inhibiting incorporation of heme to hemozoin. The mutant pfCRT protein also binds verapamil and desipramine resulting in reversing of the resistance induced by the mutant pfCRT (Bellanca *et al.*, 2014; Lakshmanan *et al.*, 2005). However, the challenge of using these chemo-sensitizers is that a higher concentration is required to achieve optimal reversal effect and this higher concentration is associated with more adverse effects, seemingly because they are already established drugs acting on different receptors for different functions (Martin *et al.*, 1987).

Since the mutant pfCRT resistance can be reversed by binding to other compounds, we hypothesized that developing a chloroquine analogue with a higher binding affinity for the protein could be co-administered with the parent compound itself (chloroquine) (Kim *et al.*, 2019; Patel and Roy, 2021). This in turn will lead to competitive racing for the binding site and subsequent elimination from the vacuole. The chloroquine analogue with a high affinity and binding capacity for protein will competitively inhibit binding of chloroquine onto the protein, decreasing its efflux from the vacuole. As such, the concentration of chloroquine will be maintained and even elevated, ensuring maximal efficacy. Furthermore, the fact that these analogues are similarly related to chloroquine means they will also prevent incorporation of heme to hemozoin, adding to the efficacy of the parent compound.

Twenty compounds out of the ZINC database search results had a chloroquine similarity index of > 95%. Twelve of these compounds showed a higher binding affinity to the mutant pfCRT protein. Four of these twelve compounds had binding affinity less than -8.0 compared to -7.0 of chloroquine with the compound ZINC01596768 having the greatest binding strength of -8.3. This strong interaction between the protein and the analogues could be useful in ensuring competitive inhibition of pfCRT binding. The other analogues were ZINC38050614, ZINC38050617, and ZINC38050615 with binding interaction strengths of -8.0, -8.2 and -8.2 respectively.

The pharmacokinetic profile prediction showed all the twelve compounds followed Lipinski rules, had no alerts on the PAINS criteria and in terms of lead likeness, they had violated the rule of XLOGP3 > 3.5 (Daina *et al.*, 2017). They also had a high GI absorption, and thus can be formulated as oral drugs. However, three of these twelve compounds (ZINC38050614, ZINC38050617, and ZINC38050615) are predicted to be substrates of P-glycoprotein and thus, even though they had a high GI absorption, their bioavailability could be reduced due to efflux by the glycoprotein (Kwon *et al.*, 2004). All the compounds were predicted to be permeant to the blood brain barrier and could potentially cause CNS side effects. When it comes to CYP450 inhibition, all the twelve compounds inhibited the enzymes CYP1A2 and CYP26. In contrast to this, all the twelve compounds did not inhibit CYP1A2 but showed mixed results when it comes to inhibition of CYP2C19. Compounds ZINC01873617 and ZINC96331701 did inhibit CYP3A4 while the rest of the compounds did not inhibit the enzyme. The Lipophilicity log P value between 1 and 4 usually provides more optimal physiochemical properties (Benet *et al.*, 2016). Of the twelve selected compounds, compound ZINC01596768, which had the highest docking score, had a log P of 4.15 which, is slightly above the optimal range.

The synthetic accessibility score for the twelve compounds were all below 3.07, nearing one and as such were easy to synthesize in the laboratory (Daina *et al.*, 2017). Other target prediction by the SwissTargetPrediction tool showed that the compounds interacted less with receptors or enzymes within the body. The compound ZINC01596768 with the highest docking score showed a probability of more than 19% of binding to histamine N-methyl-transferase, histamine 3 receptor and Quinone reductase 2 enzyme. The remaining three compounds with docking score less than -8.0 only interacted with histamine 3 receptor at a probability of more than 19%.

Conclusions

- I. 12 out of 20 compounds showed greater binding affinity for the mutant pfCRT protein than chloroquine.
- II. ZINC01596768 had the greatest binding affinity of -8.3.
- III. ZINC01596768 on simulation had no alerts on PAINS criteria, followed Lipinski rules, had high GI absorption, permeated BBB, inhibited CYP1A2 and CYP2D6 and a log P of 4.15.

Thus, compound ZINC01596768 could potentially be co-formulated with chloroquine in the treatment of malaria in chloroquine-resistant cases.

Data availability

Underlying data

Harvard Dataverse: Insilco Screening of Chloroquine Analogues for Compounds with More Affinity for the Plasmodium falciparum Chloroquine Transporter as Potential Antimalarial Drugs, <https://doi.org/10.7910/DVN/OAU1WP> (Otieno and Walekhwa, 2022).

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

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Rajalakshmanan Eswaramoorthy

Adama Science and Technology University (ASTU), Adama, Ethiopia

The authors presented their research work titled "In silico screening of chloroquine analogues for compounds with more affinity for the Plasmodium falciparum chloroquine transporter as potential antimalarial drugs" Overall, the article is well written. However, a few issues need to be corrected before it is Approved.

Comments:

1. The article requires thorough grammar and spell check.
2. The abstract section needs to highlight the critical finding of the article (numeric values and significance).
3. The references section needs to be strengthened in all sections.
4. The authors need to provide the selected compounds' 2D structures.
5. The results section needs to revise with clarity. The authors provided only tables.
6. The rationale behind this present investigation is not clear. The introduction section needs to be revised to emphasize the motivation behind this study.
7. The methodology section needs to be revised carefully.
8. Molecular docking grid box values were not given.
9. Did the authors do blind docking validation or re-docking to confirm the active site?
10. Is there any charges added to protein? What is Kollman's charge here?
11. The conclusion needs to be revised. Highlight the critical finding and comparative results.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medicinal and Computational Chemistry, Advanced Biomaterials, Tissue Engineering and Regenerative Medicine.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 29 July 2022

<https://doi.org/10.5256/f1000research.120151.r128920>

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David A. Fidock

Department of Microbiology & Immunology, Columbia University Irving Medical Center, New York, NY, USA

This article by Otieno and Walekhwa is an interesting study that uses in silico screening to search for compounds that might bind to the *Plasmodium falciparum* chloroquine resistance transporter PfCRT and therefore prove useful to combine with antimalarial drugs to which PfCRT mediates resistance via a gain of efflux. While the study is purely computational and predictive, the findings are interesting and it is a useful starting point for further studies.

- The authors should update their introduction to cite most recent WHO data on malaria

disease burden (see numbers for the year 2020).

- How many compounds did they examine in the ZINC database?
- One important improvement to the manuscript would be to also show structures in a Figure. This would show the reader if these all share a 4-aminoquinoline ring and how structurally related they are.
- These data are predictive of binding but there is no experimental evidence to support this. The authors should list in their conclusions that the compounds were predicted to have greater binding affinity for the mutant pfCRT protein.
- The authors should also state that further studies are required to test these compounds for activity against *Plasmodium falciparum* chloroquine-resistant and chloroquine-sensitive parasites cultured in vitro.
- The authors should also indicate that further work is needed to measure the binding affinity of these compounds to purified PfCRT, as was described in the Kim *et al.* 2019 article that they cite.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Plasmodium falciparum, malaria, drug resistance, drug discovery, genetics, genomics, transporters, mode of action, therapeutics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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