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Research article

DOCKING BASED VIRTUAL SCREENING OF SOME NEW 4-AMINOQUINOLINES AGAINST *Pf*CRT

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Abstract

Background*: The development of multidrug resistance in the strains of malaria parasites makes the discovery of new antimalarial drugs still relevant. Hence, the world is continuously looking for new antimalarial chemotherapeutic options.*
Objective: *In this study, some new 4-aminoquinoline derivatives were designed and screened against the chloroquine resistant transporter protein of Plasmodium falciparum (PfCRT) by in silico technique.*
Methods: *The compounds were designed considering the pharmacophore of chloroquine using Marvinsketch. Then compounds were screened against PfCRT by molecular docking based approach using Discovery Studio 2020. Finally, the best compounds were further analyzed for their toxicity, drug-likeness, drugscores and different pharmacokinetic properties.*
Results and Discussion*: Out of the fourteen compounds, nine compounds showed better docking results (CDocker energy) than the reference drugs chloroquine and piperaquine. Different docking scores of these nine compounds also suggested the excellent binding affinity of these compounds with the target protein. The binding energies of the best docking poses of these nine compounds revealed that four compounds, namely A4, A6, A7 and A10 formed more stable protein-ligand complex than the reference drugs with binding energies -82.724, -71.015, -109.853 and -95.340 kcal/mol, respectively. All these four compounds showed similar toxicity profiles with the reference drugs, but had less drug-likeness. Overall, the four compounds had more drugscore values than the reference drugs with suitable pharmacokinetic properties for the oral route.*
Conclusion: *These findings gave four new 4-aminoquinoline derivatives with better binding efficacy and stability with the PfCRT protein of P. falciparum with good drugscore values that might overcome the drug resistance problems associated with chloroquine and piperaquine.*

Keywords: Malaria, antimalarial, 4-aminoquinoline, drug resistance, *in silico* study.

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Introduction

Malaria is still considered a life-threatening infectious disease mainly in the African and Southeast Asian regions, with yearly morbidity of around four lacs as per the World Health Organization report [1]. The development of multiple drug resistance by the malaria parasite *Plasmodium falciparum* creates problems for eradicating this disease [2]. Among the various chemotherapeutic agents, chloroquine (CQ) was a potent and safe antimalarial agent. But due to the development of resistance by the malaria parasites, it becomes less effective against the disease [3]. Among the various reasons, the mutation in the *P. falciparum* chloroquine resistance transporter (*Pf*CRT) protein is a major one for decreasing the efficacy of chloroquine [4]. Along with chloroquine, the mutation in this *Pf*CRT protein has also reduced the effectiveness of another 4-aminoquinoline category drug, piperaquine (PPQ) [5]. The global spread of this *Pf*CRT mutated strain of malaria parasite has accelerated the need for new antimalarial agents to tackle the battle against malaria [6]. In this study, we designed some new 4-aminoquinoline derivatives to increase the antimalarial efficacy and analyzed their binding efficacy and stability with the *Pf*CRT protein. The different toxicity parameters of the designed compounds, their drug-likeness and drugscore were also evaluated. Finally, various pharmacokinetic parameters were also assessed to observe their applicability through the oral route.

Materials and Methods

Design and preparation of compounds

Fourteen compounds were designed using MarvinSketch v20.4 and saved as a .sdf file format for future use. The SMILES of the compounds were loaded to Discovery Studio 2020 (DS 2020) molecular modeling software (Dassault Systèmes BIOVIA, San Diego, USA) and three-dimensional structures were generated using the 'Small Molecule' tool of the DS 2020 software. Then energy minimizations of the compounds were carried out using CHARMM-based (Chemistry at Harvard Macromolecular Mechanics) smart minimizer, which performs 2000 steps of Steepest Descent followed by Conjugate Gradient algorithm with an energy RMSD gradient of 0.01 kcal/mol [7].

Preparation of the target protein and selection of binding site

X-ray crystal structure of the target protein, *Pf*CRT (PDB ID: 6UKJ), [8] was obtained from the Protein Data Bank websites (www.rcsb.org) [9]. Before the docking study, the target protein was prepared using DS 2020 software. After loading the target, it was cleaned and prepared by the 'Prepare Protein' protocol of DS 2020. During cleaning, alternate conformations were deleted, terminal residues were adjusted and, bonds and bond orders were corrected. In addition, water molecules were removed from the structure, and co-crystal ligands were kept with the proteins in the preparation process. Finally, energy minimization of the target protein was performed using the CHARMM-based smart minimizer method at maximum steps of 200 and an energy RMSD gradient of 0.1 kcal/mol [10].

The predefined active site as reported in the PDB format was selected using the 'Edit and Define Binding Site' method under the 'Receptor-Ligand Interactions' tools of the DS 2020. The active binding site sphere of *Pf*CRT had the coordinates of X: 149.546396 Y: 169.972898, Z: 138.925039 and radius 8.4 Å (Figure 1). The validation of the binding sites and docking study was done by redocking the co-crystal ligand present in the selected active binding site [11].

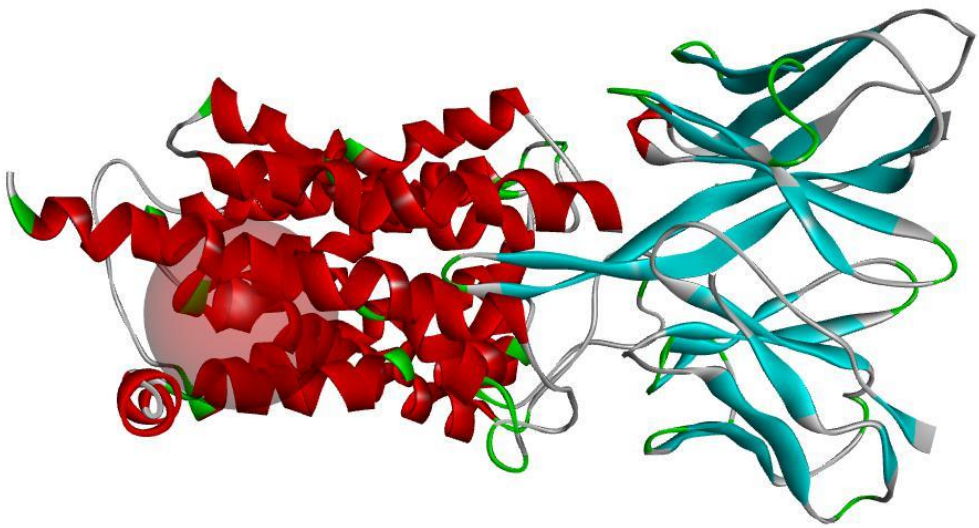

Figure 1: The selected target protein *Pf*CRT with the active site highlighted as a red sphere.

Molecular docking and scoring study

The compounds library was docked with the target using simulation-based docking protocol 'CDocker' of the DS 2020. CDocker uses a CHARMm-based molecular dynamics (MD) algorithm to dock compounds into the active binding site of a receptor [12]. In the docking study, CQ and PPQ were taken as reference drugs to evaluate the designed compound's results. The compounds showed better results than the CQ and PPQ were taken for determining the different docking scores. Different scoring functions of the best pose of the compounds like LigScore1, LigScore2, -PLP1 (Piecewise Linear Potential), -PLP2 (Piecewise Linear Potential), and –PMF (Potential of Mean Force) were determined to evaluate molecular binding affinity of the compounds [13]. The scoring functions of the reference drugs were taken as a control to assess the test compounds against the two respective targets.

Determination of binding energies of the best protein-ligand complexes

The MM-PBSA based calculation of binding energy provides the stability of the formed protein-ligand complex in the docking study. The protein-ligand complexes obtained from the preliminary docking and scoring studies were further taken to calculate binding energies using the 'Calculate Binding Energy' protocol of DS 2020 with the MM-PBSA method [14, 15].

Determination of toxicity, drug-likeness and drugscore parameters

The best compounds obtained from the binding energy calculation study were further analysed for different toxicities like tumorigenic, mutagenic, reproductive effective, and irritant. They were further analyzed for drug-likeness and drugscores using ORISIS Data Warrior 5.5.0 [16].

Determination of pharmacokinetic parameters

The selected compounds were analyzed for ADMET (absorption, distribution, metabolism, elimination, and toxicology) parameters using the 'ADMET Descriptors' tool of the DS 2020. In the ADMET analysis, different pharmacokinetic parameters like aqueous solubility, blood-brain barrier penetration (BBB), gastrointestinal absorption, cytochrome P450 (CYP2D6) inhibition,

hepatotoxicity, plasma protein binding (PPB), n-octanol/water partition coefficient (AlogP98), 2D polar surface area (PSA_2D) were determined [17].

Results and Discussion

Basis of the designed compounds

The structure-activity relationship (SAR) studies on 4-aminoquinolines suggest that 7-chloro and 4-amino groups in the quinoline nucleus are essential for high antiplasmodial and antimalarial activities. When other groups replaced the 7-chloro group, the decreased activities were noted, mainly due to the change of the pKa of the quinoline nitrogen atom. The variable alkyl chain is essential not only for circumvention of the CQ resistance mechanism but also for lipophilicity, which is a vital parameter for the effectiveness of iron-chelating antimalarials [18]. In our study, we designed our compounds by replacing the one hydrogen atom with some mono- and bi- heterocyclic groups at -R position (Figure 2).

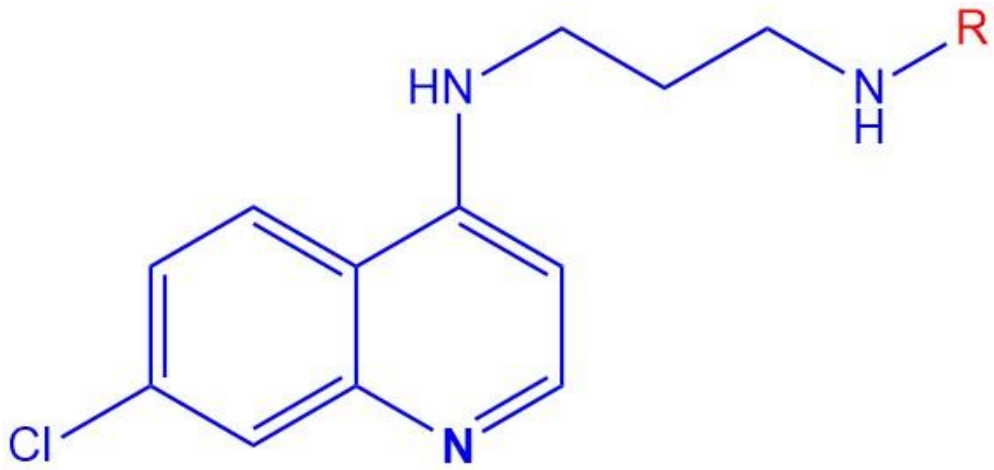

Figure 2: The parent scaffold used for designing the compounds.

Molecular docking and scoring study

In the docking study, primarily CDocker energy (kcal/mol) was considered to select the compounds having good binding affinity in the active site of the target protein in comparison to the reference drugs CQ and PPQ. More negative the CDocker energies more favorable binding affinities of the compounds with the target protein in the active site.

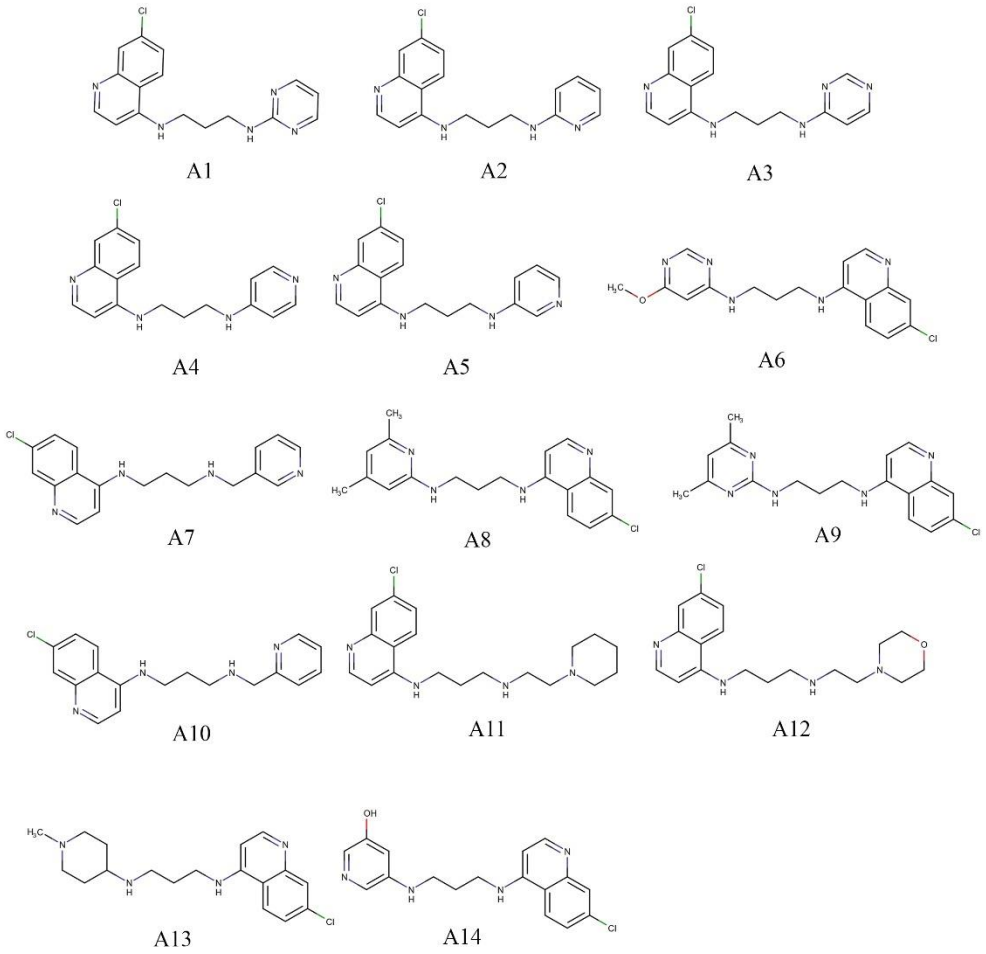

Figure 3: The new 4-aminoquinoline compounds designed for the study.

From the preliminary screening, out of the fourteen compounds, nine compounds showed better results than the reference drugs CQ and PPQ (Table 1). The best poses of these nine compounds were selected for further calculations of different docking scores. Among the different docking scores, LigScores accounts for polar attraction, -PLP evaluates hydrogen bond interaction and -PMF computes Helmholtz free interaction energies. After calculating the docking scores, it was found that the test compounds also had similar kinds of docking score values as the reference drugs CQ and PPQ. From this information, it was found that the nine compounds selected based on CDocker energies had similar kinds of binding affinities towards the target protein as the reference drugs CQ and PPQ.

Table 1: Docking and scoring results of the compounds and reference drugs.

Name	CDocker energy (kcal/mol)	LigScore1	LigScore2	-PLP1	-PLP2	-PMF
A1	-33.241	3.49	5.73	88.65	80.75	66.7
A2	-28.959	3.35	5.73	88.5	83.32	68.79
A3	-30.753	2.93	5.53	83.39	80.04	81.12
A4	-27.377	3.55	5.82	86.84	80.48	71.62
A5	-24.432	2.91	5.47	80.09	77.49	78.12
A6	-34.816	3.09	5.79	88.24	82.5	82.99
A7	-29.756	3.11	5.72	89.23	87.79	75.56
A8	-34.194	3.54	5.98	96.38	88.82	81.94
A9	-38.452	2.85	5.67	89.03	82.75	97.01
A10	-30.948	2.96	5.69	82.55	79.87	76.32
A11	-25.339	2.81	5.86	83.48	79.49	78.49
A12	-23.788	3.12	5.91	88.84	85.8	81.77
A13	-23.757	2.83	5.95	89.72	82.95	78.12
A14	-24.725	3.63	5.92	84.23	85.42	94.93
CQ	-26.686	2.72	5.59	79.97	78.66	74.81
PPQ	-10.547	3.25	6.35	87.86	86.09	111.73

Binding energy calculations

The best poses of the nine compounds obtained from the docking study were further analyzed for determining the binding energies of the protein-ligand complexes to observe the stability of the compounds in the active site. After determining the binding energies, it was found that only four compounds, namely A4, A6, A7 and A10, had the lower binding energies in comparison to the reference drugs CQ and PPQ. These compounds' lower binding energies suggested that they formed more thermodynamically stable complexes with the target protein in contrast to the reference drugs.

Table 2: MM-PBSA based binding energies of the best poses.

Name	Binding energy (kcal/mol)
A4	-82.7244
A6	-71.0158
A7	-109.853
A10	-95.3409
CQ	-68.3966
PPQ	-61.0859

Finally, interactions of the best four compounds and the reference drugs were generated to observe the interactions of the compounds with the different amino acid residues of the active site (Figure 1). The compound A4 formed six hydrophobic interactions (Alkyl, Pi-Alkyl, and Pi-Sigma) with Ile61, Phe340, Thr344, Ile347, Tyr391 and Arg392; and one charged interaction (Pi-Cation) with Arg392. A6 formed one conventional hydrogen bond with Asn395; one carbon hydrogen bond with Ser65 and seven hydrophobic interactions (Alkyl, Pi-Alkyl and Pi-Pi- T-shaped) with Ile61, Ile66, Phe340, Ile347, Val348 and Tyr384; and two charged interactions (Pi-Cation) with Arg392. A7 formed one conventional hydrogen bond with Asn395; two hydrophobic interactions (Pi-Alkyl) with Ile61 and Phe340 and one charged interaction (Pi-Cation) with Arg392. A10 formed one conventional hydrogen bond with Asn395, four carbon hydrogen bonds with Ser65, Tyr384, Ser388 and Asn395; one hydrophobic interaction (Pi-Alkyl) with Ile61 and two charged interactions (Pi-Cation) with Arg392. CQ formed one conventional hydrogen bond with Asn395; eight hydrophobic interactions (Alkyl and Pi-Alkyl) with Ile61, Ile347, Val348, Ile389 and Tyr391; and one charged interaction (Pi-Cation) with Arg392. PPQ formed one conventional hydrogen bond with Thr344; two carbon hydrogen bonds with Asn58 and Tyr384; five hydrophobic interactions (Alkyl) with Ile61, Leu69, Ile347 and Leu381; and one charged interaction (Pi-Cation) Arg392.

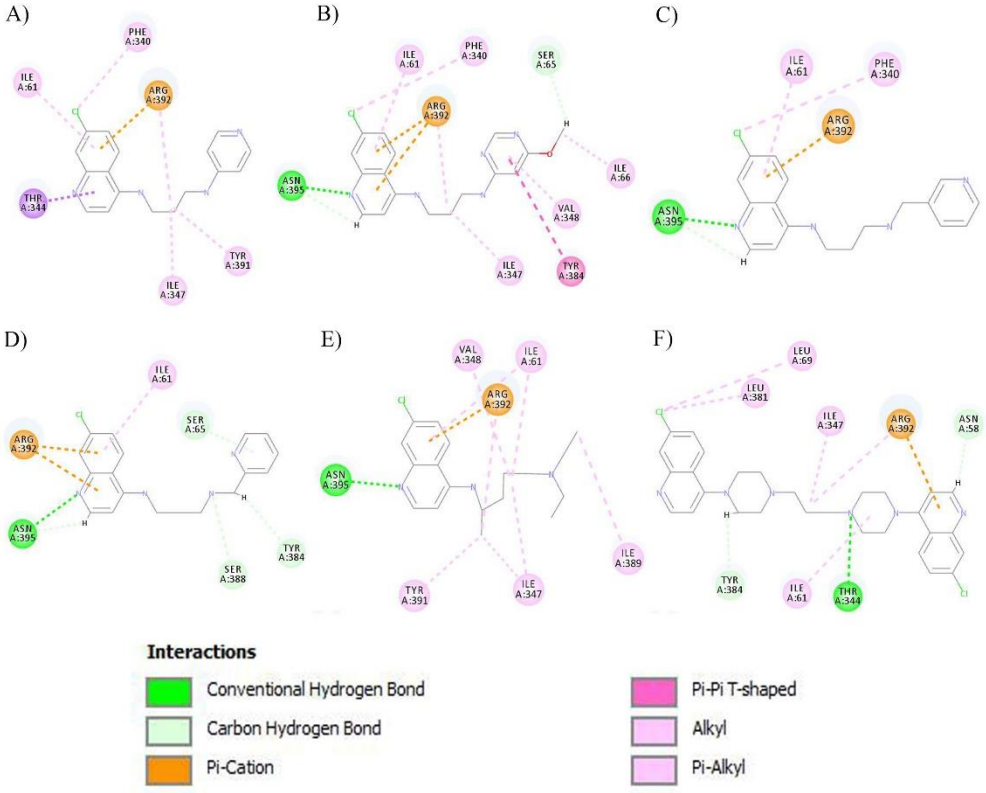

Figure 4: Different non-bond interactions of the best compounds and reference drugs with amino acid residues of the active site; A) A4, B) A6, C) A7, D) A10, E) CQ and F) PPQ.

Determination of toxicity parameters, drug-likeness and drugscores

The toxicity analysis found that all the four test compounds and the two reference drugs (CQ and PPQ) have mutagenic properties. But they all were found to be none tumorigenic and not going to have any toxic effects on the reproductive system. Other than CQ, all were found to be non-irritant. As the test drugs had similar toxicity profiles with the reference drugs, they can be considered for further investigations.

Table 3: Different toxicity parameters of the test compounds and reference drugs.

Name	Mutagenic	Tumorigenic	Reproductive Effective	Irritant
A4	high	none	none	none

A6	high	none	none	none
A7	high	none	none	none
A10	high	none	none	none
CQ	high	none	none	high
PPQ	high	none	none	none

In the drug-likeness and drugscore evaluations, the drug-likeness values of the test compounds were found to be less than the reference drugs CQ and PPQ. But the overall drugscore values of the test compounds were more than the reference drugs CQ and PPQ. Hence, it supported that the compounds can be considered for use as drugs if they show promising results in preclinical and clinical evaluations.

Table 4: Drug-likeness and drugscore values of the test compounds and reference drugs.

Name	Drug-likeness	DrugScore
A4	0.75336	0.39918
A6	0.6735	0.3441899
A7	0.33161	0.3845504
A10	0.33161	0.3828786
CQ	6.6327	0.2535989
PPQ	8.525	0.2267297

Pharmacokinetic parameters

The ADMET analysis found that the compounds A6, A7 and A10 have very good aqueous solubility (solubility level 3), but A4 has low solubility in the aqueous medium. All four test compounds have medium penetration through BBB and good intestinal absorption (absorption level 0). All four test compounds were found to be an inhibitor of CYP2D6, hepatoxic, and bind with plasma protein. The ADME plot based on the ALogP98 and PSA 2D values was generated and all four compounds were found to satisfy all the criteria.

Table 5: ADMET parameters of the test compounds and reference drugs.

Name	Solubility level	BBB Level	CYP2D6 Prediction	Hepatotoxic Prediction	Absorption Level	PPB Prediction	ADMET AlogP98	ADMET PSA 2D
A4	3	2	TRUE	TRUE	0	TRUE	2.858	48.142
A6	2	2	TRUE	TRUE	0	TRUE	3.292	68.333
A7	3	2	TRUE	TRUE	0	TRUE	2.587	48.142
A10	3	2	TRUE	TRUE	0	TRUE	2.802	48.142
CQ	2	0	TRUE	TRUE	0	TRUE	4.345	27.423
PPQ	1	0	TRUE	TRUE	0	TRUE	5.481	35.931

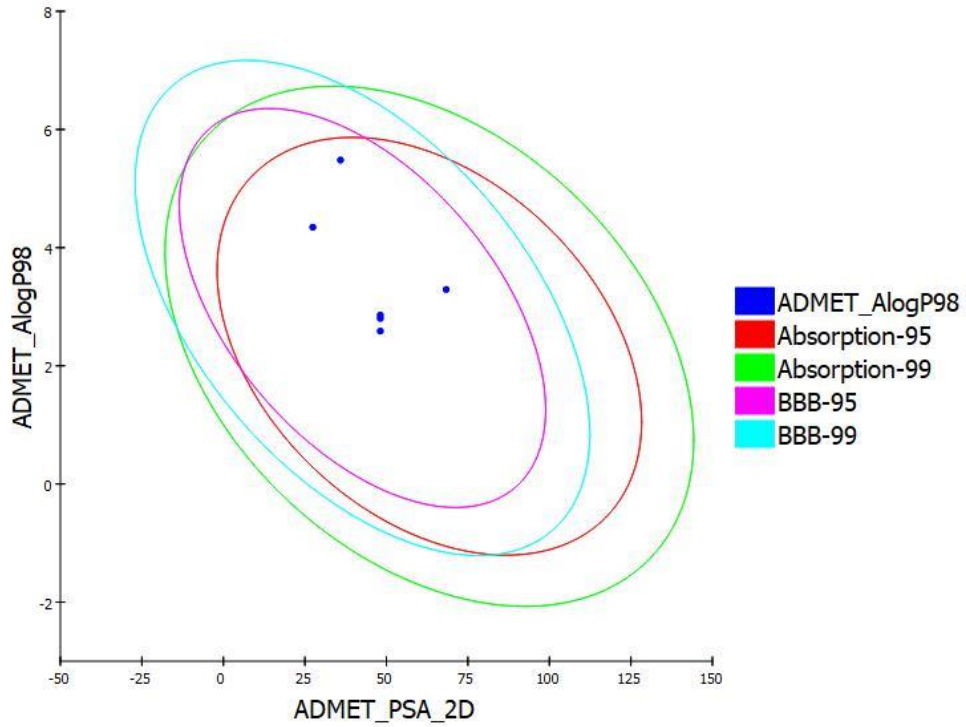

Figure 5: ADME plot of the test compounds and reference drugs.

Conclusion

Our study proposed four new 4-aminoquinoline derivatives that showed excellent binding affinities with *Pf*CRT compared to the CQ and PPQ, which have already developed resistance against the malaria parasite. Furthermore, as these four compounds formed thermodynamically stable protein-ligand complexes with *Pf*CRT, they can overcome the drug resistance developed due to the mutation of the target protein. Based on this preliminary investigation, further synthesis and *in vitro*/*in vivo* evaluations of the four compounds may provide potent antimalarial agents effective against the resistant strain of *P. falciparum*.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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