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Structure-Based Drug Design in Discovering Target Specific Drugs against *Plasmodium falciparum* Adenylosuccinate Lyase

Gbolahan O. Oduselu[1,2], Olayinka O. Ajani[1,2], Yvonne U. Ajamma[1], Ezekiel Adebiyi[1,3,4]*

[1]*Covenant University Bioinformatics Research (CUBRe), Covenant University, Ota, Ogun State, Nigeria*
[2]*Department of Chemistry, Covenant University, Ota, Ogun State, Nigeria*
[3]*Department of Computer and Information Science, Covenant University, Ota, Ogun State, Nigeria*
[4]*Division of Applied Bioinformatics, German Cancer Research Center (DKFZ), Heidelberg, Germany*

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ABSTRACT

The emergence of bioinformatics tools and methods has impressively increased the chances of the discovery of new antimalarial drugs that can act through new modes of action, with high efficacy against the deadly *Plasmodium falciparum*. An essential protein in the salvage of *Plasmodium falciparum* purines is adenylosuccinate lyase (ADSL), necessary for the synthesis of parasite's DNA, and therefore can be a potential antimalarial drug target. Hence, structure-based drug design (SBDD) was employed to screen a large dataset of compounds downloaded from the PubChem database against homology modelled *Plasmodium falciparum* adenylosuccinate lyase (*Pf*ADSL). A total of 1,082 compounds were successfully prepared using PyRX software. This was after 3,697 compounds obtained from the similarity evaluation search on 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR) were filtered with Lipinski's rule of five (RO5). AutoDock vina software was employed to perform the virtual screening against the biological target using the downloaded ligands from PubChem database with a center grid of x, y, z set on 15.930, 54.398, -5.213 and grid size of x, y, z set on 80,80, 80. A post-screening analysis showed that the five best hits from the screening possessed better binding affinities, within the ranges of -10.9 and -10.5 (kcal/mol), when compared to AICAR (-8.6 kcal/mol) and chloroquine (-6.0 kcal/mol) standards. The best hits also showed moderate toxicity and good pharmacokinetic properties. Thus, these compounds could be further validated, optimized, synthesized, and transformed into successful commercially-available antimalarial drugs.

***Keywords*:** Malaria, Drug design, Antimalarial activity, Molecular docking, Drug target, ADMET properties.

Introduction

The manufacture of a drug is a lengthy and costly procedure that takes about 12-15 years and costs approximately $1 billion.[1,2] But unfortunately, most of the proposed compounds from the discovery stages do not make it through the preclinical stages, especially because of toxicity and poor pharmacokinetics.[3] The advent of computer-aided methods of drug design (CADD) has revolutionized the discovery processes, as compound activities can be predicted even before synthesis.[4,5] Structure-based drug design (SBDD) is one of the most widely used methods in CADD. SBDD employs bioinformatics tools and methods in virtually screening large datasets of compounds for their binding affinities against the three-dimensional (3D) structure of a specific biological target.[6] Malaria is one of the life-threatening healthcare issues that affect humans, caused by protozoan parasites through the bites of infected female anopheles mosquitoes.[7,8] In 2018, an estimated 228 million cases and 405,000 deaths were recorded worldwide. Nigeria was one of the six countries that accounted for more than half of all malaria cases worldwide in 2018 with 25% of the total cases.[9] The continuous emergence of drug resistance of the malaria parasites has made the eradication of the disease more tasking.[10] This necessitates the design and development of new antimalarial drugs with different protein targets and mechanisms of action alien to the malarial parasite.[10,11] The pyrimidine and purine metabolic pathways in *Plasmodium falciparum* are different compared to those of humans, making them promising pathways for novel drug development.[12] The purine metabolic pathway enzyme adenylosuccinate lyase (ADSL) is responsible for the final step in AMP synthesis.[13] ADSL is found in the cytoplasm of *Plasmodium falciparum* and it is essential in the salvage of the parasite's purines, necessary for the synthesis of DNA.[14] Comprehensive biochemical and kinetic characterization of *Plasmodium falciparum* adenylosuccinate lyase (*Pf*ADSL) shows its significant sequence difference from the sequence of human.[13,15] Targeting *Pf*ADSL provides a promising path towards the development of novel antimalarial drugs.[12] 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) and its analogs have been proposed to have inhibitory potentials against *Pf*ADSL due to the expanded substrate sensitivity of the enzyme and there are no enzymes in *Plasmodium falciparum* that can metabolize AICAR.[13] Nitrogen-containing heterocycles have been reported to possess good pharmacological effects and the major heterocyclic backbone in AICAR is imidazole[16] (Figure 1). Also, it has been reported that the binding of AICAR plays no role in the metabolism of the parasite.[12] This study aims to determine the best hits from the structure-based virtual screening of a total of 1,082 compounds, with similar structures to AICAR, downloaded from PubChem database against homology modelled *Pf*ADSL and also

*Corresponding author. E mail: ezekiel.adebiyi@covenantuniversity.edu.ng
Tel: +2347066263787

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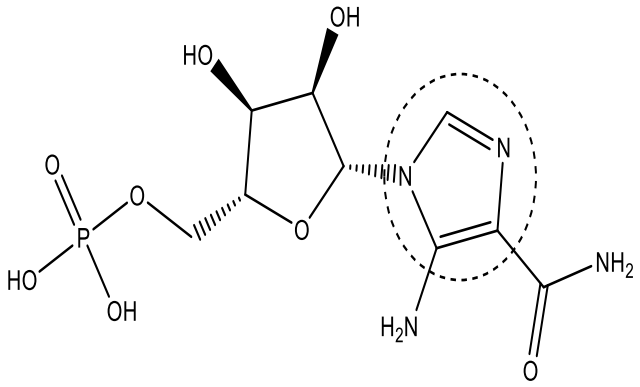

Figure 1: Structure of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR). Imidazole template is highlighted in the circle

predict their ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties. The best hits could be further validated with more complex modelling approaches such as molecular dynamics simulations, synthesized, tested *in vitro* or *in vivo*, and then processed for clinical trials.[17]

Materials and Methods

Ligand preparation

AICAR (PubChem ID: CID65110) was used as the control ligand and evaluation of similar structures with 100% search of the PubChem database gave 3,697 compounds. The 3,697 compounds were filtered using the "Lipinski rule of five", RO5. After the filter, 1,099 compounds were obtained and the compounds were downloaded in their sdf formats. The downloaded sdf formats of the compounds were opened with PyRX software.[18] All the compounds were minimized using the Universal Force Field (uff)[19], as implemented in the Open Babel software package.[20] The energy minimization for the compounds is necessary to generate 3D structures with proper bond lengths between the different atoms. Only 1,082 were successfully minimized and converted to Autodock docking formats (pdbqt) out of the 1,099 compounds due to the inability to set up a force field for some compounds.

Protein preparation

The homology model of the 3D structure of the *Plasmodium falciparum* adenylosuccinate lyase (*PfADSL*) predicted and reported in previous work[21] by our Research team was employed. The modelled *PfADSL* was edited using AutoDockTools 1.5.6 by removing the non-amino acid residues, computing the Gasteiger charges, adding polar hydrogens, and merging of the non-polar hydrogens.[22]

Virtual screening and post-screening analyses

Autodock4[23] was used to prepare the grid box (to establish the binding pocket) on the *Pf*ADSL around Arg 17A, Tyr 18A, Asn 312A, His 173C, Asn 90D, Asp 92D, Gln 250D, Arg 338D, Ser 343D, and Arg 347D.[21] A spacing of 0.375 Å was used to prepare the grid box while the center grid was set on 15.930, 54.398, and -5.213 on x, y, and z respectively. Also, the size on x, y, and z was set to 80,80 and 80. The structure-based virtual screening of the 1,082 compounds on *Pf*ADSL was carried out using Autodock vina. After the vina simulation, five best hits (compounds with the lowest binding affinities) were obtained while the hydrogen bond formations were also analyzed using Ligplot.[24] The lower the binding affinity, the stronger the binding of the compound to the binding residues.[25]

ADMET studies

One of the most significant advances in drug research in recent years has been the invention of in silico methods for predicting compound absorption, delivery, metabolism, excretion, and toxicity (ADMET).[26] ADMET studies on the compounds with the lowest binding affinities were carried out using variable nearest neighbor (vNN) webserver (https://vnnadmet.bhsai.org/).[27] This web server estimates some of the most significant components in identifying a potential drug candidate. These include the likelihood of causing drug-induced liver injury (DILI), cytotoxicity (HepG2), human liver microsomal stability (HLM), cytochrome P450 inhibition (drug-drug interactions), blood-brain barrier (BBB), P-glycoprotein (Pgp) substrate and inhibitor, human ether-à-go-go-related gene (hERG) blockers, mitochondrial membrane potential (MMP) disruption (mitochondrial toxicity), chemical mutagenicity (AMES Test), and Maximum Recommended Therapeutic Dose (MRTD).

Results and Discussion

Structural elucidation of the five best hits

Purine backbone is observed as a common core template in the structures of the five best hits. The five best hits possess amino groups on position 2 and carbonyl groups on position 6 (Figure 2).

The variations in the structures are observed on their position 9. It is important to note the positions of the functional groups, as this will contribute greatly to the lead optimization stage.[28] It was also noted that hits with PubChem IDs: 136499047, 136678837, and 136454903 have similar chemical structures but different conformations.

Predicted compounds with the lowest binding affinities and hydrogen bond formations

The post-screening analyses showed that the five best hits possess better binding affinities, within the ranges of -10.9 and -10.5 (kcal/mol), when compared to AICAR (-8.6 kcal/mol) and chloroquine (-6.0 kcal/mol) standards (Table 1).

Compound with PubChem ID: 137283912, was observed to possess the best binding affinity (-10.9 kcal/mol) amongst the 1,082 compounds used for the virtual screening. Also, residues of the homology modelled *Pf*ADSL that form hydrogen bonds with the five best hits, control ligand, and chloroquine are shown in Table 1.

The hydrogen bonds formed in the docking model validates the structural and functional stabilities of the ligand-protein complexes.[29] It has also been reported that slight alteration in the conformation of ligands can lead to a significant difference in the docking score and geometry of the binding poses, for flexible docking simulations.[30]

In this study, we observed a slight difference in the binding affinities of the hits with PubChem IDs: 136499047, 136678837, and 136454903, which possess similar chemical structures but different conformations. Also, the hits with PubChem IDs: 136499047, 136678837, and 136454903 formed hydrogen bonds with similar amino acid residues but with different bond lengths. Compound (PubChem ID: 137283912) with the strongest binding in the docking model formed hydrogen bonds with Gln250D, Gly297A, Ser299A, Asn306A while the control ligand (AICAR) formed hydrogen bonds with Tyr18A, Asn90D, Asp92D, Asn312A, Arg338D, Ser343D, Arg347D (Figure 3).

ADMET properties of the compounds with the lowest binding affinities

The results of the ADMET properties of the top five hits of the structure-based virtual screening showed that the compounds have relatively fair pharmacokinetics and toxicity according to the vNN ADMET model. Compound with PubChem ID: 137283912, for example, is predicted to have a tendency of hepatotoxicity, no tendency of cytotoxicity, will undergo human liver membrane metabolism, and will not inhibit the activity of human cytochrome P450. Furthermore, the predictions show that the compound is unable to cross the blood-brain barrier (BBB), will not be a P-glycoprotein inhibitor or substrate, will not block the hERG gene nor disrupt the mitochondria, and will not cause chemical mutagenicity.

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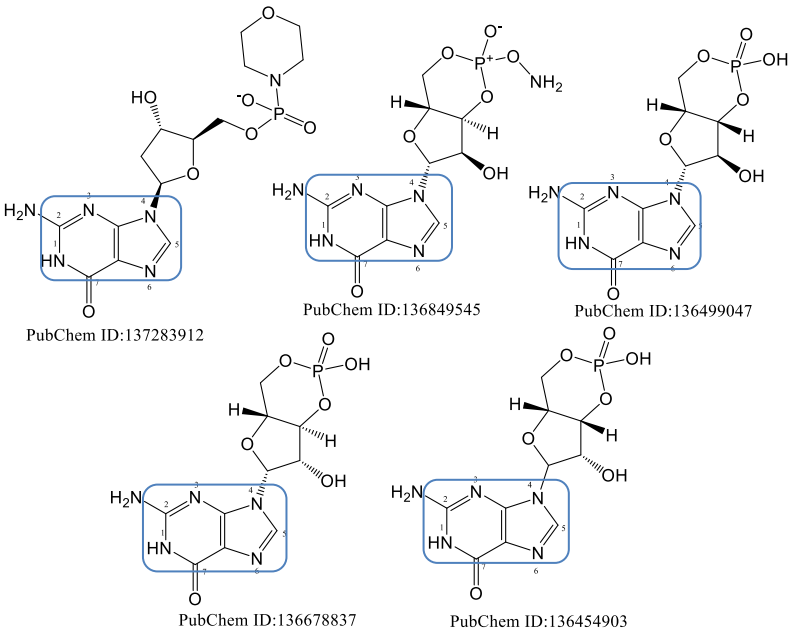

Figure 2: Structural elucidation of the five best hits. The scaffolds in the blue boxes show the purine core template common to all the five best hits.

Table 1: The molecular weights, binding affinities, and residues of the homology modelled *Pf*ADSL that form hydrogen bonds with the five best hits, control ligand, and chloroquine

S/N	PubChem ID	MW g/mol)	BA (kcal/mol)	Residues that form hydrogen bonds
1	137283912	415.32	-10.9	Gln250D, Gly297A, Ser299A, Asn306A
2	136849545	360.22	-10.7	His91D, His173C, Gln250D, Ser299A, Asn306A, Ser343D
3	136499047	345.21	-10.6	Tyr18A, Asp92D, Asn312A, Ser343D, Arg347D
4	136678837	345.21	-10.6	Asp92D, Asn312A, Ser343D, Arg347D
5	136454903	345.21	-10.5	Tyr18A, Asp92D, Asn312A, Ser343D, Arg347D
6	Control ligand (AICAR)	338.21	-8.6	Tyr18A, Asn90D, Asp92D, Asn312A, Arg338D, Ser343D, Arg347D
7	Chloroquine	319.90	-6.0	-

*MW = Molecular weights, BA = Binding affinities

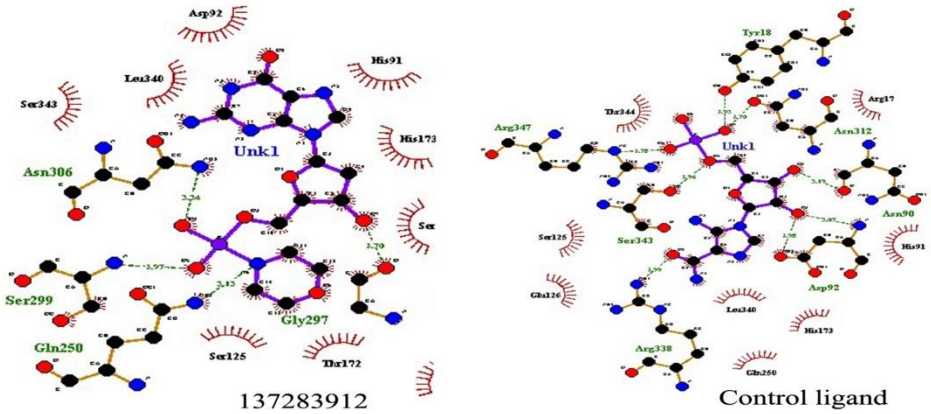

Figure 3: Post-screening visualization of the hit, PubChem ID: 137283912, and control ligand in the binding pocket of homology modelled *Pf*ADSL. The hydrogen bonds are indicated with the green dash lines.

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Table 2: The ADMET results of the compounds with the lowest binding affinities

	Liver Toxicity		Metabolism		Membrane Transporters				Others		
PubChem 1D	DILI	Cyto-toxicity	Cyp HLM	Cyp Inhibitor	BBB	P-gp Inhibitor	P-gp Substrate	hERG Blocker	MMP	AMES	MRTD (mg/day)
137283912	Yes	No	Yes	No	No	No	No	No	No	No	158
136849545	Yes	No	Yes	No	No	No	No	No	No	No	171
136499047	Yes	No	Yes	No	No	No	No	No	No	No	148
136678837	Yes	No	Yes	No	No	No	No	No	No	No	148
136454903	Yes	No	Yes	No	No	No	No	No	No	No	148

DILI, drug-induced liver injury; CYP, cytochrome P450; HLM, human liver microsomes; BBB; blood-brain barrier; Pgp, P-glycoprotein; hERG, human ether-a-go-go-related gene; MMP, mitochondrial membrane potential; AMES; chemical mutagenicity; MRTD, maximum recommended therapeutic dose.

Conclusion

Structure-based drug design steps including ligand library design and preparation, receptor preparation, binding site identification, virtual screening, and post-processing analyses have been carefully carried out in this project. A total of five hits with good binding affinities, orientations, and better activities than the known hit (AICAR) of the target protein have been reported. [(2R,3S,5R)-5-(2-amino-6-oxo-1H-purin-9-yl)-3-hydroxyoxolan-2-yl]methoxy-morpholin-4-ylphosphinate, with PubChem ID: 137283912, was observed to possess the best binding affinity (-10.9 kcal/mol) amongst the 1,082 compounds used for the virtual screening. Also, it was established that conformational changes in the ligand structures had an impact on the binding affinities of the ligands and the geometry of their binding poses. The ADMET studies showed that the compounds have good pharmacokinetics and toxicity, therefore, can be considered for hit-to-lead ideation. Further validations and lead optimizations are encouraged before the synthesis and development of these compounds into active commercial antimalarial drugs. The experimental characterization of the protein target should be carried out to validate its 3D crystal structure.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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