Molecular Dynamics Simulation and Analysis of some Ligands on *Var2csA* Target

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Abstract

Prevalence of malaria during pregnancy and the spate of drug resistance by malaria parasites have constantly impacted maternal, perinatal and neonatal outcomes, especially in sub-Saharan Africa. Inhibiting binding; or displacement of bound infected erythrocytes from the placenta as an adjunct treatment or vaccine for malaria was considered an option towards ending pregnancy associated malaria in this study. Molecular modeling and toxicity predictors used in this study indicated that among the ligands screened, IH3 had the lowest binding energy of -9.8Kcal/mol while var2csA had -2.8Kcal/mol. Var2csA is parasite's adhesive protein. It was also observed that out of the 90 ligands (binding affinity range -9.8 to -1.0 Kcal/mol) screened, IH3 (-9.8Kcal/mol), FAD (-8.4 Kcal/mol), NDP (-8.2 Kcal/mol), A5A (-8.2 Kcal/mol), ABO (-8.1 Kcal/mol), IH2 (-7.8 Kcal/mol), 2RT (-7.7 Kcal/mol), CRO (-7.7 Kcal/mol) and IH1 (-7.7 Kcal/mol) appear to be the most promising lead compounds to occupy var2csA binding pocket in pCSA in order to prevent adhesion of malaria infected erythrocytes to the placenta. SwissADME and Molinspiration Cheminformatics for LogP (mean of 1.07 and range of -2.79 to 4.18) of the lead compounds showed no correlations between lipophilicity and interaction with receptors. Of all the compounds selected for analysis, only ABO and 2RT exhibited drug-like properties based on Ghose, Lipinski and Veber filters. The data therefore suggests that IH3, FAD, NDP, A5A, ABO, IH2, 2RT, CRO, IH1 and var2csA make favourable lead candidates for targeting pCSA and therefore require further in vitro and in vivo evaluations.

Keywords: Malaria; Pregnancy; Modeling; Toxicity; Auto Dock

Abbreviations: pRBCs: Parasitized Red Blood Cells; IEs: Infected Erythrocytes; DC: Domain Cassette; EPCR: Endothelial Protein C Receptors, PAM: Pregnancy Associated Malaria; DARC: Duffy Antigen Receptor for Chemokines; EC: Endothelial Cell; IPT: Intermittent Preventive Treatment; PDB: Protein Data Bank; TEST: Toxicity Estimation Software Tool; FAD: Flavin Adenine Dinucleotide

Introduction

Background to the Study

Malaria is one of the leading causes of death in tropical, underdeveloped countries throughout the world. Malaria in pregnancy is a major global public health concern; this has been attributed to transient depression in cell-mediated immunity which has increased the susceptibility of pregnant women to malaria and its adverse outcomes. Approximately 32.3% of pregnant women in sub-Saharan Africa are at the risk of malaria yearly [1]. Malaria is caused by *Plasmodium* sp transmitted by the bite of the female *Anopheles* mosquito. Upon infection, the life cycle of the *Plasmodium* sp. begins and ends in the bloodstream where erythrocytes are infected and consequently destroyed, causing symptoms such as anemia, respiratory sequelae, cerebral malaria, metabolic acidosis, and eventually organ failure, leading to death [2]. However, before the total destruction of erythrocytes occurs, there seems to be a mode by which the parasitized

red blood cells (pRBCs) become sequestered or adhered in particular sites throughout the vascular system [3] and this results in widespread sequestration of infected erythrocytes (IEs) and hence their reduced clearance from the blood stream by the spleen [4]. *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) mediates this adhesion of IEs to various host receptors on the vascular lining during the blood stage of malaria infection [4-6].

Proteins of the PfEMP1 which include var2csA (DBL2x, DBL3x and DBL6e), Var1csA (DBL3y), CIDR groups B & C, CIDR of DC8 group A, CIDR of DC13, DC4, DC8, DC5, Duffy Binding Protein (DBP) for Pv & Pk, and PfRh4 for Pf, PfRh5, EBA - 175, EBL - 1, EBA - 140 and EBA 181 mediate adhesion and cell invasion through specific binding to multiple endothelial cell (EC) receptors, including domain cassette (DC) 36, DC31 also known as platelet endothelial cell adhesion molecule 1 (PECAM -1) or cluster of differentiation 31 (CD31) [7], DC13, DC8, intercellular adhesion

molecule -1 (ICAM - 1), E-selectin, endothelial protein C receptors (EPCR), duffy antigen receptor for chemokines (DARC), and placental chondroitin sulfate A (pCSA). Others include: CD36, complement receptor - 1 also known as CD35 (i.e.; C3b, or C4b), VLA-4, LW, CD 58, CD 147, CD44, B-CAM/LU and Glycophorin A (F actin and G actin).

In this study, we focused on pregnancy associated malaria (PAM) also referred to as placental malaria (PM) which is mediated by *Plasmodium falciparum* elaborated *var2csA* that binds to placental chondroitin sulfate A (pCSA) to bring about sequestration of malaria IEs on the placental syncytiotrophoblast.

Each *Plasmodium falciparum* parasite genome constitutes over 59% *var* genes [5,8] and individual parasite expresses a single *var* gene at a time [9], maintaining all other *var* genes in a transcriptionally silent state. Almost all members of the *var* family are classified into one of 3 major groups (A, B, and C) based on a combination of chromosomal location, transcription direction, and upstream promoter sequence [10]. As an important activity in many drug discoveries projects, in silico molecular modeling was employed to identify molecules with activity against pCSA and subsequent lead optimization of the hits identified [11].

Statement of the Problem

Use of insecticide treated net and intermittent preventive treatment (IPT) are currently the preventive strategies to improve maternal and fetal outcomes amidst the ravaging malaria scourge. However, due to waning efficacy of the IPTs and insecticide treated nets as a result of resistant strains of the parasite and the malaria transmitting vectors, these preventive measures have become ineffective and less reliable over-time. This problem has persisted for a while now and there is no valid vaccine yet to prevent malaria, especially, placental malaria [12].

In this study, our target is to identify “in silico”, for the purpose of clinical application, non-recombinant product(s) that can competitively inhibit *Plasmodium falciparum* infected erythrocytes (PflEs) from binding, or displace bound PflEs from placental chondroitin sulfate A since no antibody or antigen based vaccine tend to be successful in the search for vaccine against pregnancy associated malaria.

Aim and Objectives of the Study

Aim

This study aims to discover “in silico” non recombinant molecules which can interact with placental chondroitin sulfate to inhibit binding or displace bound *var2csA* from the placenta in order to prevent pregnancy associated malaria.

Objectives

1. To generate a library of molecules similar to *var2csA* (ligands to CSA) from available databases.
2. Dock these molecules against placental CSA in order to generate hits with favorable binding affinity with it; and

3. Analyze “in silico” the suitability and safety of some of the selected molecules as drugs.

Operational Definition of Terms

1. Autodock: Molecular modeling simulation software. It is especially effective for protein–ligand docking.
2. Binding affinity: The strength of the binding interaction between a single biomolecule to its ligands.
3. Databases: A collection of information that is organized so that it can be easily accessed, managed, and updated. Example here include PDB and PubChem.
4. Virtual molecular screening (also known as molecular docking): A computational technique used in drug discovery to identify those structures which are most likely to bind a drug target, typically a protein or enzyme.

Literature Review

Concept of Drug Design and Receptor Interaction

Many micro and macroscopic properties of substance, such as biological activities of drugs and drug-like compounds are controlled by their stereochemistry. Molecules with one or more rotational degree of freedom (rotatable bonds) exhibit conformational isomerism, where the stable conformers correspond to energy minima, and therefore, to the most probable structures to be found in materials, pharmaceuticals and agrochemicals [13]. Interaction stabilizing conformations are provided by certain hydrogen bonding even though others also take place in many cases, as seen in electrostatic effects and hyperconjugation phenomena, i.e, electron donation from the endocyclic oxygen lone pair toward the vicinal axial antibonding orbital [14]. For example, the core of the CSA-binding site is situated in the N-terminal part of *var2csA* by hydrogen bonding and van der Waal force (Figure 1). The following equation $[EI]_{aq} \rightleftharpoons [E]_{aq} + [I]_{aq}$ is used to determine the reaction involving a receptor and its ligand. The change in free energy of binding (ΔG) is related to binding affinity, ($\Delta G = -RT \ln K_A$).

$$K_A = \frac{[EI]}{[E][I]}$$

Where E = Receptor, I = Ligand, K_A = Equilibrium constant, R = ideal gas constant = 8.314 J/mol-K, and T = Temperature of the reaction (Kelvin).

Receptor and ligand docking aim at correct prediction of the structure of the complex under equilibrium conditions. The docking techniques used in this study included prediction of the binding modes of novel drugs (ligands) and the study of protein-protein interactions using AutoDock Vina (<http://vina.scripps.edu>) tool in PyRx (<http://pyrx.sourceforge.net>) where autodock reproduces nine different protein - ligand complexes for which the structure of the macromolecule was known without prior knowledge of the binding site. The more negative the binding affinity becomes, (i.e, the lower the binding energy), the better the orientation of the ligand in the binding pocket of the receptor.

The autodock programme package - PyRx was able to select the correct complexes based on the energy without prior knowledge

of the binding site; (i.e, blind docking). PyRx was not able to see the binding site but was able to find it.

Figure1: Receptor- Ligand Pose.

Prediction of correct structure (posing) of the $[EI]$ complex does not require information about k_A . Prediction of biological activity (ranking) does require information about K_A . For the complex $[EI]$, the following factors are important: steric, electrostatic, hydrogen bonding inhibitor strain (if flexible) and enzyme (receptor) strain. Important factors when considering the equilibrium include: desolvation, rotational entropy, and translational entropy. Most scoring functions focus on energetic than entropic effects. The events of ligand-binding are driven by a combination of enthalpic and entropic effects, either of which can

dominate specific interactions [15-17].

Var2csA

Var2csA is a variant of many adhesive proteins of *Plasmodium falciparum* erythrocyte membrane protein 1 (*PfEMP1*) elaborated on the surface of *Plasmodium falciparum* infected erythrocytes for the purpose of tethering infected cells on tissue receptors to evade destruction by the spleen. *Var2csA* protein consists of six DBL domains, a single transmembrane helix, cytoplasmic acidic terminal sequence and significant interdomain regions (Figure 2). Four of the DBL domains have been shown to bind CSA [18-20].

Figure2: Image of the DBL domains [21].

The structure of the DBL3X domain has been shown [22,23] to have an α -helical scaffold similar to those observed in DBL domains from the invasion proteins like erythrocyte-binding antigen 175 (EBA-175) and the Duffy antigen receptor for chemokines (DARC) - binding protein [24,25].

It has also been shown that full length recombinant *var2csA* binds specifically to CSA with nanomolar affinity, and that the CSA- binding site lies in the N-terminal part of the protein [26]. The core CSA- binding site lies within the DBL2X domain and part of the flanking interdomain regions, in contrast to the idea that single domains do not possess the structural requirement

for specific CSA binding. They added that the x-ray scattering measurement revealed CSA- binding DBL2X domain is situated in the center of the structure.

Materials and Methods

Materials

The following computing resources and items were used:

Compounds: The protein data bank (PDB) structure of the receptor chondroitin sulfate A (PDB ID: 3bqk) in its raw and prepared forms is shown in Figure 4. Structures of some of the ligands used are shown in Figures 3.

Figure 3: Image of the Ideal SDF Formats of some Ligands.

Figure 4: PDB formats of 3bqk.

Hardware and Software: This work was carried out on windows 8.1 Pro with processor: Intel® Core (TM) i5 CPU M 520 @ 2.40GHz having installed memory (RAM): 4.00GB (3.86 GB usable) on system type: 64 bit operating system although 32-bit Windows

Vista operating system also work well. PyRx docking software version 0.8 for Windows [14]. PyRx is open source software to perform virtual screening. It is a combination of several softwares such as AutoDock Vina, AutoDock 4.2, Mayavi, Open Babel, etc.

PyRx uses Vina and AutoDock 4.2 as docking softwares. In this study, AutoDock Vina [27] was used. Discovery studio (Discovery Studio: v20.1.0.19295), Pymol (Pymol stereo 3D quad buffer) and ICM Browser (Molsoft MolBrowser 3.8-7d) were used to examine structural properties and study binding interaction between receptor residues and ligands. SwisADMET, and Molinspiration cheminformatics were used for evaluation of drug-likeness of the ligands and Toxtree [28] was additionally used to determine the toxicity and safety profile of the ligands.

Databases and Applications: Protein data bank (rcsb.org) and PubChem (https://pubchem.ncbi.nlm.nih.gov/) were used to download the chemical structures of the receptor (3bqk) and those of the ligands. Canonical SMILES and other information about the ligands and the receptor were extracted from PubChem. Toxtree, Toxicity Estimation Software Tool (TEST), SwissADMET, Molinspiration and Lazar Toxicity Predictor were employed to test

various toxicity and safety parameters of lead compounds.

Methods

Retrieval of Macromolecule: The structure of the macromolecule-chondroitin sulfate A (CSA) was retrieved by searching in the protein data bank (PDB) (rcsb.org/structure/3bqk); downloaded and saved as a PDB format.

Retrieval of Liands: Molecules constituting the ligands screened belong to the same enzyme commission (EC) class of CSA and were downloaded from rcsb.org as Excel file. In the file, the ID values of all the class members were copied and pasted on a page “download ligand” site in the PDB in order to generate their corresponding ideal structure data file (SDF) formats. The “launch download” application of PDB downloaded and channeled the ligands into a folder (ligands) that was created on the desktop. Some of the ligands are presented in Table 1.

Table 1: Some of the ligands to 3bqk (CSA).

Ligand ID	Ligand Formula	Ligand MW	Ligand Name
13P	C3 H7 O6 P	170.06	1,3-DIHYDROXYACETONEPHOSPHATE
A5A	C13 H19 N7 O7 S	417.4	‘5’-O-(N-(L-ALANYL)-SULFAMOYL) ADENOSINE
ADP	C10 H15 N5 O10 P2	427.2	ADENOSINE-5’-DIPHOSPHATE
BAB	C17 H19 N8	335.39	BIS(5-AMIDINO-BENZIMIDAZOLYL) METHANE
FAD	C27 H33 N9 O15 P2	785.55	FLAVIN-ADENINE DINUCLEOTIDE
GTP	C10 H16 N5 O14 P3	523.18	GUANOSINE-5’-TRIPHOSPHATE
IH3	C31 H35 N7 O3	553.66	2-(2,2-DIPHENYL-ETHYL)-7-METHYL-1,3-DIOXO-2,3,5,8-TETRAHYDRO-1H-[1,2,4] TRIAZOLO[1,2-A]PYRIDAZINE-5-CARBOXYLIC ACID [4-(2-AMINO-3H-IMIDAZOL-4-YL)-CYCLOHEXYL]-AMIDE
MOH	C H4 O	32.04	METHANOL
SO4	O4 S	96.06	SULFATE ION
2Y8D	C2 H7 N S	77.149	2-AMINO-ETHANETHIOL

Target Preparation and Docking Process: The x-ray structure of the receptor (3bqk) was downloaded from protein data bank (rcsb.org), cleaned from hetatoms in discovery studio and resaved (Figure 4). The input ligand files were also prepared for virtual screening by converting them to PDBQT file format when they were imported into PyRx software as chemical table file (SDF). Following a series of steps, the library of the ligands was docked into the active site of 3bqk in the PyRx platform using Amber Van der Waals in the active box of 17 x 15 x 17. Autodock Vina took each ligand and bonded its different conformations to the receptor molecule (3bqk) to get the binding energies in different orientations of each ligand. Each ligand has nine different binding orientations starting from 0 to 8.

Validation of Docking Process: Docking was repeated three times on the same system specifications for the purpose of process validation and all returned minimal variation (P<0.01 data not shown) in uff energy, binding energy and RMSD values.

Binding Analysis: Discovery studio (Discovery Studio: v20.1.0.19295), Pymol (Pymol stereo 3D quad buffer) and ICM Browser (Molsoft MolBrowser 3.8-7d) were all used to analyze binding interactions between residues of 3bqk and the ligands.

Drug likeness: Toxtree, Toxicity Estimation Software Tool (TEST), SwissADMET, Molinspiration and lazarus toxicity predictions were variously employed to test toxicity and safety parameters of lead compounds.

Results and Discussion

Results

Analysis of Docking Result: A library of 90 compounds in the same EC class with chondroitin sulfate A, known to interact with pCSA was generated from rcsb.org. Compounds in the library demonstrated good binding affinity with many having higher binding affinity to pCSA (3bqk) than 2y8d a var2csA molecule and SO₄, a functional species in chondroitin sulfate A (Table 2).

Table 2: Comparison of Binding affinities of some ligands to pCSA from autodock output.

S/N	Autodock Output	PyRx Binding Affinity (Kcal/mol) on 3bqk
1	3bqk_IH3_uff_E=898.86	-9.8
2	3bqk_FAD_uff_E=1533.84	-8.4
3	3bqk_A5A_uff_E=1074.29	-8.2
4	3bqk_NDP_uff_E=1972.57	-8.2
5	3bqk_AB0_uff_E=394.68	-8.1
6	3bqk_IH2_uff_E=686.03	-7.8
7	3bqk_2RT_uff_E=579.67	-7.7
8	3bqk_CRO_uff_E=438.90	-7.7
9	3bqk_IH1_uff_E=1030.16	-7.7
10	3bqk_SO4_uff_E=428.16	-3.7
11	3bqk_2Y8D_uff_E=43.4	-2.8

The first 75 ligands and SO₄ have binding affinity higher than that of 2Y8D. This implies that *var2csA* can be displaced from pCSA in preference for molecules which have higher binding affinity. 2Y8D and SO₄ which have their highest binding affinity as -2.8Kcal/mol and -3.7Kcal/mol also ranked 719th and 655th respectively. For this study, only the first nine ligands with the

highest binding affinity (-9.8 to -7.7Kcal/mol), 2y8d and SO₄ were selected for the target analysis. The complex formed for some of the ligands are represented in figure 5, the complexes have different structural modification from the SDF format seen in Figure 4 except for SO₄ which it is not a valid selection for binding analysis. It is not a chain, molecule or a multiple residue object.

Figure 5: Auto Dock Structure files of some ligands.

Analysis of Binding Interactions: Receptor residues interacting with SO₄, 2y8d and the top nine selected ligands as seen in Discovery studio and Pymol are listed in Table 3a.

Evaluation of Drug likeness and Organ Toxicity of some Selected Hits: The lipophilicity for selected compounds is as shown by the values of their consensus logP in Table 4. Drug likeness of the compound's Tables 5a & 5b.

Discussion

All the ligands in this study yielded their lowest binding energy at their first binding modes (0). The reliability of this study was

ascertained by triple - docking of the ligands with the receptor (pCSA) and it was found that both the first, second and the third dockings maintained the same values in all parameters and for all binding modes ($P < 0.01$ data not shown), giving IH3 and FAD as the first two ligands with the lowest binding energy as shown in table 2 and implicitly, the ligands of choice to bind placental chondroitin sulfate during in vitro and in vivo evaluations. The binding affinities of the top nine compounds on *var2csA* target are closely related that they lie within a close range of -9.8 to -7.7Kcal/mol indicating that their potential as lead compounds for 3bqk is comparable. Auto Dock reproduces nine different protein -

ligand complexes for which the structure of the macromolecule was known, without prior knowledge of the binding site but here only the result from the first binding orientation was presented since it is the one with the highest binding affinity, the full binding orientations and their RMSD values for all the ligands are available on request. The RMSD for the first binding orientation is always 0. The binding affinity and the RMSD value collectively determine the ranking of the molecules.

Analysis of Binding Pattern

Considering their binding interactions, FAD had the highest

number of favorable hydrogen bonds with receptor residues but shares equal number of hydrophobic contacts with IH3 (Table 3a & Table 3b). FAD is a drug already in used to treat eye diseases caused by vitamin B2 deficiency, such as keratitis and blepharitis (Drugbank, <http://www.drugbank.ca/>). Some other compounds like NDP, ABO, IH2, CRO, IH1 and SO₄ possess no hydrogen bonds but have good number of other hydrophobic contacts except CRO and SO₄ which do not have any significant favourable bonds with receptor residues based on observation from Discovery Studio (Figures 6).

Figure 6: 2D view of the interactions between lead compounds and pCSA (3bqk) residues. (a) 2-(2,2-diphenyl-ethyl)-7-methyl-1,3-dioxo-2,3,5,8-tetrahydro-1h-[1,2,4] triazolo[1,2-a]pyridazine-5-carboxylic acid [4-(2-amino-3h-imidazol-4-yl)-cyclohexyl]-amide (IH3-9.8Kcal/mol), (b) Flavin-adenine dinucleotide (FAD-8.4 Kcal/mol), (c) NADPH dihydro-nicotinamide-adenine-dinucleotide phosphate (NDP-8.2 Kcal/mol) and (d) 2-amino-ethanethiol (2Y8D-2.8 Kcal/mol).

Table 3a: Binding Affinities and Receptor-Ligand interactions.

S/N	Compound	Binding affinity (Kcal/mol)	Hydrogen bond interaction with Residues	Hydrophobic bond interaction with Residue
1	IH3	-9.8	ASP A:1353, LEU A:1272	VAL A: 1363, TYR A:1354, TPR A:1453, SER A: 1362, ASN A:1356, THR A:1269, ASN A:1271, GLN A:1270, TPR A:1278, GLY A:1284, GLY A: 1283. MET A:1357, ILE A:1369, VAL A:1274, TYR A:1366. HIS A:1368. GLN A:1449.
2	FAD	-8.4	HIS A:1345, ASN A:1542, ASN A:1337, GLN A:1348,	GLU A: 1545, LYS A: 1341, GLU A:1459, GLY A:1342, ASP A:1335, CYS A: 1344, PRO A: 1336, CYS A:1437, GLU A:1436, TYR A:1543, ARG A:1349, ILE A:1352, SER A: 1452, PRO A:1442, THR A:1443. LYS A:1455. PRO A: 1544.
3	A5A	-8.2	TYR A:1565, SER A:1513	LYS A:1562, GLN A:1517, ILE A:1512, LYS A:1509, TYR A:1561, PHE A:1553, GLY A:1568, ASN A:1556, ILE A:1554, PHE A:1555, ASP A:1557. LYS A:1516.

4	NDP	-8.2	*	CYS A:1251, TPR A:1249, ILE A:1265, GLN A:1270, TYR A:1366, GLU A:1367. LYS A:1252. ILE A:1250, ILE A:1365.
5	ABO	-8.1	*	PRO A:1336, PRO A:1544, GLU A:1545, ASP A:1335, LYS A:1335, LYS A:1341, LYS A:1333, ASN A:1325, GLY A:1342, HIS A:1345, GLY A:1332.
6	IH2	-7.8	*	ILE A:1265, ILE A:1250. 1LE A:1365. LYS A:1252.
7	2RT	-7.7	LYS A:1341, GLU A:1545	HIS A:1345. LYS A:1333. GLU A:1436, PRO A:1336, GLU A:1459, PRO A:1544, CYS A:1437, CYS A:1344, ASP A:1335, GLY A:1342.
8	CRO	-7.7	*	LYS A:1247, TYR A:1366, ILE A:1365, ILE A:1250. LYS A:1252.
9	IH1	-7.7	*	LYS A:1525, ALA A:1263, TRP A:1249, TRP A:1453, PHE A:1454, VAL A:1451, HIS A:1257.
10	So ₄	-3.7	*	*
11	6058/2Y8D	-2.8	SER A:1450, CYS A:1264, GLN A:1522.	LYS A:1525, ALA A:1263, TRP A:1249, TRP A:1453, PHE A:1454, VAL A:1451, HIS A:1257.
*value not available				

Table 3b: The number of Favorable Intermolecular Interactions of Ligands with Receptor.

S/N	Complex	No. of Hbonds	No. of Hydrophobic contacts
1	3bqk_IH3_uff_E=898.86	2	17
2	3bqk_FAD_uff_E=1533.84	4	17
3	3bqk_A5A_uff_E=1074.29	2	12
4	3bqk_NDP_uff_E=1972.57	*	9
5	3bqk_ABO_uff_E=394.68	*	11
6	3bqk_IH2_uff_E=686.03	*	4
7	3bqk_2RT_uff_E=579.67	2	10
8	3bqk_CRO_uff_E=438.90	*	*
9	3bqk_IH1_uff_E=1030.16	*	5
10	3bqk_SO4_uff_E=428.16	*	*
11	3bqk_6058/2Y8D_uff_E=43.4	3	7

The binding affinities for the top nine compounds are closely related (ranging from -9.8 to 7.7Kcal/mol) which implies the likelihood of similar potential as lead compounds to bind 3bqk. 2y8d is a molecule of var2csA, the parasite protein of interest which is the reference protein. Conventional bonds are grouped together into hydrophobic bonds while every other bond interaction is grouped together into hydrophobic bond. 2D interactions between receptor residues and some selected hits were presented in figures 6.

Drugability of Compounds

No correlation was observed between lipophilicity and the interaction with the receptor by considering cLogP values and number of conventional bonds (Tables 3b & Table 4). However, for FAD, NDP and A5A, interaction with the receptor is correlated with low lipophilicity while 2RT has high cLogP value. The compounds with high cLogP values used their polar functional groups to interact with the receptor. FAD, 2Y8D and IH3 exhibited good

hydrogen bond interaction with *var2csA* receptor of placental chondroitin sulfate A possibly due to their polarity.

Screening the compounds for drug likeness using Lipinski, Veber and Ghose filters, FAD, A5A and IH1 did not satisfy the requirements for a safe drug in all the filters used. However, only ABO and 2RT suitably satisfied the conditions for drug likeness in all the filters and therefore likely the safest (Table 5b). Notwithstanding these observations, all the compounds selected

can be relatively safe since the unsatisfied molecules tend to violate 1 or 2 conditions in 1 or 2 of the three filters. Again, FAD which violated almost all the conditions in all the three filters is already in the market for other medical uses.

Based on toxicity prediction of some compounds in Lazar Toxicity Predictor, FAD, NDP and ABO penetrate the human blood

brain barrier (Table 6) indicating possibility of central nervous system toxicity. But this could also be an advantage for the compound to access the brain to elicit their pharmacologic activity in the event of susceptible cerebral malaria. They also tend to be non-carcinogenic in mouse model based on observations from the same platform.

Table 4: Comparison of cLogP values for the selected compounds as seen in SwissADMET and Molinspiration platforms.

S/N	Compound	SwissADMET cLogP	Molinspiration cLogP	Mean cLogP
1	IH3	3.08	2.65	2.87
2	FAD	-2.89	-2.69	-2.79
3	NDP	*	-5.7	*
4	A5A	0.16	-0.21	-0.03
5	ABO	0.04	-0.12	0.04
6	IH2	3.01	2.68	2.85
7	2RT	3.47	4.88	4.18
8	CRO	-0.48	-2.75	1.62
9	IH1	1.8	0.43	1.12
10	SO ₄	*	-4.16	*
11	2Y8D	0.03	-0.56	-0.27
cLogP = octanol/water coefficient.				

Table 5a: Druglikeness of selected compounds based on Lpinski, Veber and Ghose rule.

S/N	Compound	Mw (g/mol)	TPSA (Å²)	No of HBA	No of HBD	No of RB	cLogP
1	IH3	553.67	132.73	4	3	8	3.08
2	FAD	785.55	382.55	20	9	13	-2.89
3	NDP	0	0	*	*	0	*
4	A5A	969.04	344.92	15	8	18	0.16
5	ABO	344.36	103.68	8	2	5	0.04
6	IH2	526.63	127.9	4	3	9	3.01
7	2RT	330.39	86.52	4	2	4	3.47
8	CRO	319.31	136.45	7	4	5	-0.48
9	IH1	581.48	170.42	6	3	9	1.8
10	SO ₄	96.06	88.64	*	*	0	*
11	2Y8D	77.15	64.82	1	1	1	0.03

Table 5b: Druglikeness of selected compounds based on Lpinski, Veber and Ghose rule continued.

S/N	Compound	Lipinski Filter	Veber Filter	Ghose
1	IH3	Yes; 1 violation: MW>500	Yes	No; 3 Violations: MW>480, MR>130, #atoms>70
2	FAD	No; 3 violation: MW>500, NorO>10, NHorOH>5	No; 2 Violations: Rotors>10, TPSA>140.	No; 4 Violations: MW>480, WLOGP<-0.4, MR>130, #atoms>70
3	NAD	*	*	*

4	A5A	No; 3 violation: MW>500, NorO>10, NHorOH>5	No; 2 Violations: Rotors>10, TPSA>140.	No; 3 Violations: MW>480, MR>130, #atoms>70.
5	ABO	Yes; 0 violation	Yes	Yes
6	IH2	Yes, 1violation: MW>500	Yes	No; 3 Violations: MW>480, MR>130, #atoms>70.
7	2RT	Yes; 0 violation	Yes	Yes
8	CRO	Yes; 0 violation	Yes	No; 1 violation:WLOGP<-0.4
9	IH1	No; 2 violation: MW>500, NorO>10	No; 1 Violations: 1 TPSA>140	No; 3 Violations: MW>480, MR>130
10	SO ₄	*	*	*
11	2Y8D	Yes; 0 violation	Yes	No; 4 Violations: MW>160, MR>40, #atoms>20

Further insight into ABO and 2RT shows that they are better drugs, having “Yes” in all and many other rules investigated. Many other compounds down the column with higher rank than 2y8d can also be found to be good drugs (data not shown). FAD is already an FDA approved drug for the treatment of keratitis. Even though it has the second highest binding affinity with 3bqk, it appeared to have very poor drug likeness based on Lipinski, Veber and Ghose rules. ABO and 2RT are the safest among the selected compounds based on Lipinski, Veber and Ghose rules (Table 5b).

MW: Molecular weight, TPSA: Total polar surface area, No of HBA: Number of Hydrogen Bond Acceptors, No of HBD: Number of Hydrogen Bond Donors, No of RB: Number of Rotatable Bonds, cLogP: mean cLogP values, *: Not valuable values,

Table 6: Toxicity Prediction of some Compounds as seen in Lazar Toxicity Predictor Platform.

S/N	Compound	Blood Brain Barrier Penetration (Human)	Carcinogenicity (Mouse)	Maximum Recommended Daily Dose (Human)
1	IH3	N/A	N/A	N/A
2	FAD	Penetrating	Non-carcinogenic	0.000819mmol/kg-bw/day
3	NDP	Penetrating	Non-carcinogenic	0.643 (mg/kg_bw/day)
4	A5A	Penetrating	N/A	0.00178 (mmol/kg-bw/day)
5	ABO	N/A	N/A	1.32 (mg/kg_bw/day)
6	IH2	N/A	Carcinogenic	N/A
7	2RT	N/A	N/A	N/A
8	CRO	N/A	N/A	N/A
9	IH1	N/A	N/A	N/A
10	SO ₄	N/A	N/A	N/A
11	2Y8D	N/A	N/A	N/A

N/A: Data not available.

Summary of Findings, Conclusion, and Recommendations

Summary of Findings

In this study, an extensive search was carried out in silico to discover non recombinant substances that could serve as vaccines or adjunct treatment for pregnancy associated malaria (PAM) also known as placental malaria (PM). Ligands (or small molecules or drugs) with activity against chondroitin sulfate were extracted from designated drugs and proteins databases. The ligands were screened against a receptor (pCSA, PDB ID: 3BQK) to isolate molecules that can favourably bind it thus displacing or inhibiting binding of *var2csA*, a *Plasmodium falciparum* (*Pf*) protein usually

deposited on the surface of *Pf*-infected erythrocytes that enhances the adhesion of the parasite carrying cells to the endothelium of tissue vasculature, thereby tethering them away from splenic destruction.

Ninety ligands were screened out of which 75 were found to have higher binding affinity with pCSA than *var2csA*, with binding affinity ranging from -9.8 to -2.8 (Table 2, complete data not shown). Out of this number, the best nine ligands (IH3, FAD, NDP, A5A, ABO, IH2, 2RT, CRO and IH1,) whose binding affinities ranged from -9.8 to -7.7 were isolated for further in silico evaluations. Their analysis showed that four compounds (IH3, FAD, A5A and 2RT) had hydrogen (strong covalent) bond interaction with 3bqk while five (NDP, ABO, IH2, CRO and IH1) had no hydrogen bond

with 3bqk. One compound (CRO) does not have both hydrogen and hydrophobic bonds with 3bqk, yet it has high binding affinity with the receptor. This was possible because, the compound may have used its polar functional groups to make the interaction. The same is applicable to SO₄ (a sulfate ion) which also do not have any hydrogen or hydrophobic contact with the receptor. 2Y8D had both hydrogen bond and hydrophobic bond interactions with the receptor (Table 3a & Table 3b).

In silico drug ability testing of the 9 selected compounds including SO₄ and 2y8d indicates that only four compounds (ABO, 2RT, CRO, and 2Y8D) based on Lipinsky, Veber and Ghose rules perfectly satisfied drug likeness conditions (Table 5b). That means 2Y8D can be administered to mimic the parasitic form of it in binding placental chondroitin sulfate A. FAD violated all the rules for a safe drug but it has been approved for use in the treatment of eye diseases caused by vitamin B2 deficiency, such as keratitis and blepharitis. Lazar Toxicity Predictor (Table 6) showed FAD, NDP and ABO to have ability to cross blood brain barrier and non-carcinogenic.

Conclusion

Following the analysis and observations so far, all the 9 selected compounds including SO₄, 2y8d and all the rest of the compounds screened deserve further in vitro and in vivo evaluations to determine which compound has the best in vivo activity against placental malaria and safe for human use.

Recommendations

This study therefore recommends the repurposing and extending the use of flavin adenine dinucleotide (FAD) to cover prophylactic management of malaria among pregnant women since it does not require much further safety evaluations, and accelerated evaluation of the entire screened compounds to compare efficacy and safety among them.

Limitations of the Study

Chondroitin sulfate A has so many similar compounds (3BQK, 1C4S, 3BQL, 3BQI, 3CML, 3CPZ, 2Y8D, and 4P1T) which have different molecular conformational structures that may impact on the outcome of the investigation carried out. This implies the likelihood of targeting a wrong target in silico. However, this observed factor may have very minimal impact on the outcome as the compounds show slightly altered binding affinity with some of the different molecular conformations of the chondroitin sulfate A when tried, (data not indicated).

Contribution to Knowledge

Valid execution of the foregoing recommendations in this study without hitting the expected success satisfies the quest to continue or discontinue our search in this direction else, the study remains inevitable.

Suggestions for Further Studies

1. Molecular Docking and Analysis, and in vitro / in vivo evaluations on other PfEMP1 proteins targets.
2. Invitro studies of clinical outcomes of use of flavin adenine dinucleotide (and other ligands) in placental, cerebral and severe malaria.

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