Computer Assisted Design and in-Silica Metabolic Exploration of a Hemoglobin Docking Molecule- Early Steps of a Novel Antiplasmodial Agent

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Abstract

This report presents the early steps in the development of a novel antimalarial agent. Using bio-informatics, human hemoglobin was targeted for docking a ligand at pre-specified regions of its three dimensional structure. Wide ligand search from databases identified atenolol type structures as undesirable ligands that docked close to the heme binding site while Rifampin type structures were identified to dock at the target. Structure analysis was used to develop a library of potential ligand and N-[cyclohexyl (pyrrolidine-2-yl) methyl] naphthalene-2-amine was identified as the lead molecule.

Introduction

The Orphan Drug Discovery Study (ODDS) group of Usmanu Danfodiyo University, Sokoto, Nigeria, is a research team formed with a vision to discover drugs that utilize novel targets in diseases that are common in developing economies. Some of these diseases are often classified as orphaned because of low commercial interest. The cost of early phase of drug development may be reduced significantly by making use of the high computational powers now obtainable and exploiting database of receptors, enzymes and ligands also available as open resources. Malaria remains a huge burden on global health and Africa bears over 85% of this burden, which is likely to increase with the on-going evolution of climate [1]. Vector control and drug therapy are the key methods that have been employed to mitigate morbidity and mortality from malaria. Artemisinin congeners and quinine are the more efficacious anti-plasmodia agents but like all previous anti-malarial, reduced sensitivity and resistance are now being reported [2]. Newer anti-malarial with novel mechanism of action are obviously desirable but the poor commercial returns from malaria drug discovery make this of little commercial interest to Pharmaceutical companies. Our group therefore chose the discovery of anti-malarials as one of its priorities. This thesis reports the early phase of the design of a potential antimalarial molecule.

Methods

Target identification: The pre-erythrocyte cycle of the plasmodium falciparum (and plasmodium malarie) is a non-redundant unidirectional process that is target-rich. The parasite is particularly vulnerable at the erythrocytes stage because it would be essentially harmless to man without this stage. The red cell cycle produces about 6-32 merozoites per red cell per day [3]. Interrupting this cycle appears a reasonable target. Parasite entry and metabolism are obvious options but the unique nature of erythrocyte membrane is not yet fully elucidated and current knowledge of intra-erythrocyte parasite metabolism is far ahead of entry mechanism. For successful intra-erythrocytic asexual reproduction, 70-80% of resident hemoglobin is consumed [3] though only a fraction of the amino acids are utilized [4]. It appears that the excess digestion prevents premature red cell lysis [5, 6]. Though, available successful anti-malarial exploit hemoglobin digestion pathway of parasite, none has attempted at focusing on the globin part. The protein, globin, portion could be vulnerable targets because parasite reproduction is not efficient in patients with genetic abnormalities of the globin protein. Although the precise mechanism by which heamoglobinopathies ameliorate malaria is unknown, it is obviously linked to non-fatal perturbation of the globin molecule as a result of mutation [7]. Hemoglobin was therefore chosen as the drug target on the hypothesis that docking a small molecule within the tetramer of globin well away from the active heme site should not affect oxygen carrying capacity but may perturb its utility to plasmodia parasite for development. The challenge was therefore to identify a docking site in the hemoglobin globule and use its molecular characteristic to design a ligand.

Drug design: Using Chemaxon, RCSP PDB Ligand
explorer 3.8, Discovery Studio Visualizers, the structures of oxygenated, deoxygenated and native human hemoglobin were downloaded from the protein database (PDB) using appropriate identifiers and were then explored for possible docking sites. The structure of G-glycoprotein coupled receptor was also downloaded as representative of other classes of peptide macromolecule for negative screening. The intermolecular space between the 4 chains (Arrow in Illustration 1) was selected as the docking target because this will then require one ligand to influence all chains. Using Discovery Studio visualizer’s molecule window and sequence window alternatively, amino acids and atoms around this site were mapped out. The characteristic of this docking site (so called molecular lock) that may be used in ligand design (so called molecular key) include steric and electrostatic complementarities as well as hydrophobic interactions. These same characteristic may be used as constraints for unwanted structures when docking at certain part of the target molecule has been specified as undesirable. In the case under consideration, docking at the haeme- globin interaction site and at oxygen docking sites was considered undesirable. The absolutely conserved histidine amino acids that bind to heme iron in hemoglobin were specifically constrained.

**Metabolic exploration & development of molecule Library:** Metabolizer 5.3.6 software was used to explore all possible in-vivo metabolites of the designed molecule using human phase I and II reaction library in-silico with the software set to use the major pathway method. Metabolic exploration in bacteria, rats, mouse and plant species were also performed using the major pathways. Major metabolites from all these reactions were identified and analysed. All those that meet the initial constrain (stearic, electrostatic and hydrophobic) were selected and saved into a ligand molecule window and sequence window alternatively, amino acids and atoms around this site were mapped out. The characteristic of this docking site (so called molecular lock) that may be used in ligand design (so called molecular key) include steric and electrostatic complementarities as well as hydrophobic interactions. These same characteristic may be used as constraints for unwanted structures when docking at certain part of the target molecule has been specified as undesirable. In the case under consideration, docking at the haeme- globin interaction site and at oxygen docking sites was considered undesirable. The absolutely conserved histidine amino acids that bind to heme iron in hemoglobin were specifically constrained.

**Docking evaluation of ligand library on Hemoglobin Molecule & Lead molecule identification:** The primary design and metabolites from the library were docked onto hemoglobin molecule in the deoxy, oxy and native state and examined for fitness and affinity using Marvin View, Dock 6 and Ligand explorer software. All those that fit were also docked onto GCR. Ligands that specifically dock onto the targeted hemoglobin site without interaction with the haeme region were selected for lead molecule optimization.

**Lead molecule optimization:** Ligands that dock to or within 10 Angstroms of the target and also remote from the haeme binding sites in all oxidation states of hemoglobin were selected. Pharmacophore exploration was then done using Discovery studio visualizer. The pharmacopores were then used as fixed moieties within backbones of various structures of known pathways of synthesis. This ensures that the final ligands are synthesizable and stable. The final ligands selected were also optimized for octane –water partition coefficients (logP) and dissociation coefficients (logD) at PH ranges 1.5, 5.0, 6.5 and 7.4 representing the possible ranges from the stomach to the plasma. Finally the best fit ligand was blasted onto pubchem to confirm uniqueness.

**Results**

The targeted docking site is as shown in Illustration 1. Characteristics of atoms around the heme binding site of hemoglobin were determined as shown in Illustration 2. Atenolol was therefore suggested as an existing ligand that could dock close to the heme site and was confirmed to be so (Illustration 3). Structures similar to atenolol were thus excluded and new structures constrained within this limit. Rifampin was identified as the largest existing ligand that docks to the target site (Illustration 4). The lead molecule designed was therefore as shown in Illustration 5, with preferred IUPAC name as:

\[ N-[cyclohexyl (pyrrolidine-2-yl) methyl] naphthalene-2-amine. \]

It is synthesizable by sequential digestion of penicilllin G, first by beta-lactamase enzyme, adding pyrrole ring, then amidase digestion followed by adding naphthalene ring. Its dissociation curve (logD) at various PH is as shown in Illustration 6. The value at PH 1.5 and 7.4 are 0.51 and 1.71 respectively. The Octane-water partition coefficient (logP) was 4.71 and Pka analysis revealed distributions of its micro species are as shown in Illustration 7. No isoelectric point was identified (Illustration 8). Conformation analysis revealed the lowest energy conformer as in Illustration 9, with energy of 64.66Kcal/mol. Topology analysis revealed steric effect indices, distance degrees and eccentricity as shown in Illustration 10 a, b and c respectively. Major metabolic pathway analysis in man predicted that it undergoes aliphatic hydroxylation to:

\[ N-[(R)-cyclohexyl (4, 5-dihydro-1H-pyrrol-2-yl) methyl] naphthalen-2-amine \]

and then alpha aliphatic carbon hydroxylation to:

\[ (3S)-5-[(R)-cyclohexyl [(naphthalen-2-yl) amino] methyl]-2, 3-dihydro-1H-pyrrol-3-ol yl] amino] methyl]-2, 3-dihydro-1H-pyrrol-3-ol \]

Each of these metabolites also docked to the target...
site. The libraries of potential ligands obtained from combinatorial methods with 6-aminopenicillioic acid backbone contained 152 structures.

**Discussion**

This study showcases the ability to target a particular area in the 3 dimensional crystallographic structure of a protein and dock a ligand. Denovo design of molecule is possible but runs the risk of obtaining unstable or non-synthesizable structure. In this regard exploring pre-existing ligands, as in this study, for favourable scaffolds should be more reassuring. All procedures in this study have been in-silico using bio-informatics. Its strongest point is that known patterns of reactions in human have been used and may, therefore, be more predictive of final outcome on real term trials than animal models. The lead molecule identified revealed distribution coefficients that suggest that absorption of oral formulation is feasible and will most likely occur at the lower small intestine because a coefficient of 0.51 in gastric type PH suggest that most of the drugs remain in solution within the stomach. The octane –water partition coefficient is encouraging and predicts a large volume of distribution. Furthermore, significant entry into red cells appears plausible. The food vacuole of plasmodia is highly acidic. This suggests that the lead molecule may cross the lipid membrane of the vacuole and get trapped within due to its distribution pattern at acid PH. The next challenge is to use the lead molecule as a backbone for structures that modify the 3D conformation of hemoglobin sufficiently to interfere with digestion by the parasite. This may be done by synthesizing the molecules defined in the library above using a rapid screening method like plasmodium bergei rat models [8]. Hits may then be further analysed and optimized.

**Conclusion(s)**

Early attempts at drug design using bioinformatics in the 1980s did not meet much success due to the limited computational powers available at that time. Modelling, therefore, had to be based on many assumptions. New age computers have powers consistent with robust modelling with little or no assumption. This study has revealed a scaffold for hemoglobin targeting but it is still at very early stage.

**Abbreviation(s)**

ODDS: Orphan drug discovery study group, RCSP: Research Collaboratory for Structural Bioinformatics , PDB: Protein Data Base, logP: Octane-water partition, logD:Distribution coefficient, PH: acidity, Pka: acid dissociation constant, 3D: Three dimensions

**Authors Contribution(s)**

Bello Shaibu Oricha conceived and designed the research, conducted the modelling and wrote the manuscript Shuaibu Abdulmalik conducted the modelling, acquired the structures of the protein macromolecules and participated in writing the final manuscript

**Reference(s)**

Illustrations

Illustration 1

Hemoglobin showing the targeted docking site (arrow) and the 4 hemes

Illustration 2

Characteristics of atoms around heme
Illustration 3

Atenolol docks near the heme site

Illustration 4

Rifampin docks at the target site
Illustration 5

Lead Molecule

Illustration 6

Distribution of the lead molecule
Illustration 7

Distribution of microspecies of the lead molecule

Illustration 8

Evaluation of isoelectric point of lead molecule
Illustration 9

Lowest energy conformation of lead molecule
Illustration 10

Topology analysis of lead molecule (a) Stearic effect indices, (b) Distance degree, (c) Eccentricity
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