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Molecular Docking Investigation of New Inhibitors of *Falciparum vivax*

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Abstract

Despite the vigorous research and development, as of 2017, there is currently no widely available antimalarial vaccine. An effective, commercially available vaccine would be a huge game changer; however, it seems like there is still a long way to go until that target is reached. Therefore, the purpose of this study was to use molecular docking technique to identify new inhibitors for a novel antimalarial target with the overall aim of finding hit compounds which could be further optimized to become potential drug candidates. The docking protocol AutoDockVina was used alongside the molecular visualisation software UCSF Chimera to dock 100 naphthoquinones (labelled TM1-100) and 66 aryl diketones (labelled TM101-166) with the chosen target, *Plasmodium vivax* N-myristoyltransferase (*Pv*NMT). Each docking session yielded the best 9 binding modes between the ligand and target. The hydrogen bond interactions of all binding modes were analysed, and the top six target molecules (TM) were short listed as the possible hit compounds (TM40, TM65, TM66, TM81, TM94 and TM165). These compounds displayed more than six hydrogen bonds under 3 angstroms over the 9 binding modes. Using Lipinski's rule of 5, the potential hit compounds were further analysed to determine the drug-likeness and all were found to obey the parameters. Following the same method used to dock the ligands, twelve FDA approved antimalarial drugs were also docked with *Pv*NMT for comparison purposes. Apart from proguanil, the other eleven antimalarial drugs displayed fewer hydrogen bonds under 3 angstroms over the 9 binding modes compared to all six of the potential hit compounds. This study discovered six compounds which displayed stronger interactions with the target protein compared to majority of the FDA approved drugs. The results of this investigation gave us new molecules that could be further investigated for the designing of novel drug-like compounds for the treatment of Malaria.

Keywords

Falciparum vivax, Hydrogen Bond Interaction, Molecular Docking, Lipinski's Rule, N-Myristoyltransferase (PvNMT)

1. Introduction

A total of 91 countries were considered malaria-endemic in 2016 which is 17 countries less than in the year 2000 [1]. The number of fatal malaria cases has also decreased by more than 40% since 2000 for all World Health Organisation (WHO) regions [2]. Despite the incredible reductions, malaria continues to be the world's deadliest parasitic disease. An estimated 216 million cases of malaria occurred globally in 2016 alone, including 445,000 fatal cases [1]. A substantial portion of these cases (194 million) were reported in the African region which has been the case for many years. Children, the elderly and pregnant women are at most risk of contracting malaria [3]. This is evident as the majority of the fatal cases were reported to be children under the age of five [1].

1.1. Aetiology

Malarial infection is caused by protozoan parasites belonging to the genus *Plasmodium.* There are five species of *Plasmodium* (*P*), *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi.* Of the five types, *P. falciparum* is the most dangerous and is responsible for majority of the global malarial deaths [1] [3] However, the most abundant type is *P. vivax* with exposure to approximately 2.5 billion humans across the globe [4].

Plasmodium successfully thrives due to its lethal life cycle. The completion of this cycle is dependent upon two factors, the female Anopheles mosquito and humans [5]. According to Centers for Disease Control and Prevention [5], Plasmodium life cycle can be divided into 12 stages, these stages are summarised below:

Plasmodium transmission occurs at stage 1 where an infected mosquito penetrates the human skin for a blood meal [3] [5]. Within 30 minutes, the parasite travels from the saliva of the mosquito to the host's liver cells, where it begins to rapidly reproduce and eventually enter the blood stream to invade red blood cells, stages 2 - 6 [5]. A portion of the parasites invading the red blood cells develop into separate sex cells called gametocytes which are taken up by another mosquito during a blood meal, stage 7; this ultimately leads to the production of numerous parasitic cells in the mosquito's salivary gland, stages 8 - 12, thus continuing the life cycle of the malarial parasite [5].

Approved antimalarial drugs exert their effects at different stages of this life cycle with the overall aim of diminishing *Plasmodium's* abundance within the human host. Antimalarial agents will be discussed in detail later.

Despite the vigorous research and development, as of 2017, there is currently

no widely available antimalarial vaccine [6]. This is due to a combination of challenges such as the complex nature of *Plasmodium's* life cycle, and the ever-changing antigens presented by the parasite [7]. An effective, commercially available vaccine would be a huge game changer; however, it seems like there is still a long way to go until that target is reached.

1.2. Current Antimalarial Agents

Antimalarial agents alone can reduce the incidence and prevalence of malaria, and they remain at the forefront of malaria control [8] [9].

These drugs can be categorized into their two main therapeutic roles. Firstly, prompt use of these drugs not only treats an individual but also indirectly stops the development of gametocytes (stage 7 of the Plasmodium life cycle) hence blocking the spread of malaria. Secondly, antimalarial agents can be used as a preventative measure for people visiting or residing in endemic areas [9]. **Table 1** displays the current available drugs grouped into their respective drug classes.

1.3. Issues Surrounding Antimalarial Drugs

Although antimalarial drugs have a wide clinical application, they are surrounded by many issues that question their credibility. For many years, chloroquine was the gold standard treatment for uncomplicated malaria, however, this has drastically changed due to the global spread of chloroquine-resistant *falciparum* and *vivax* malaria [10]. Amodiaquine **3** and piperaquine **4** have similar structural similarities with chloroquine **1**, as shown in **Figure 1**, therefore, these drugs are also subject to increasing resistance [9].

The naturally occurring quinine **5** is the oldest antimalarial drug dating back to the seventeenth century. This drug along with other quinoline-based antimalarials such as chloroquine **1** and mefloquine **6** have widely reported adverse

Table 1. Current anti malaria drugs.

Drug class	Drug name
4-aminoquinoline	Chloroquine (1)
	Piperaquine (2)
	Amodiaquine (3)
8-aminoquinoline	Primaquine (4)
	Quinine (5)
Arylamino alcohol	Mefloquine (6)
	Lumefantrine (9)
Sesquiterpene lactone endoperoxides	Artemether (11)
	Artesunate (12)
	Dihydroartemisinin (13)
Mannich base	Pyronaridine(10)
Antifolate	Pyrimethamine (8)
	Sulfadoxine (7)
Naphthoquinone	Proguanil (15)
	Atovaquone (14)

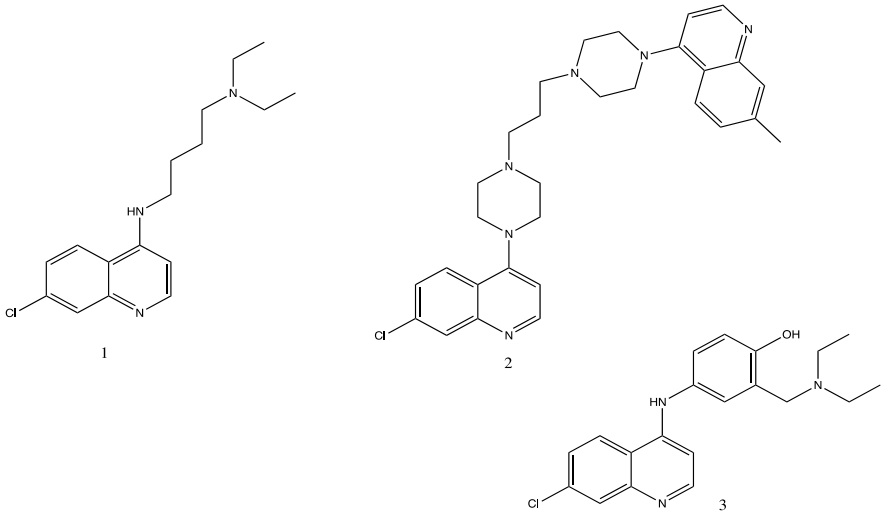


Figure 1. Chemical structures of Chloroquine **1**; Amodiaquine **2** and Piperaquine **3**.

psychiatric effects which includes sleep imbalances, mood changes, impulsivity, delusions, hallucinations and also mania; in extreme cases, suicidal thoughts and suicides were also reported [11]. Quinine has been known to be very hard to tolerate; therefore, its use is limited to severe malaria only [9].

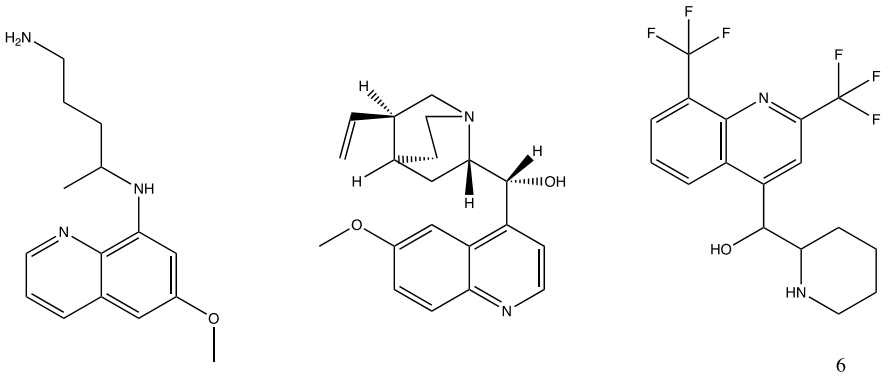
Primaquine **4** (**Figure 2**) was first licensed by the FDA in 1952 and has remained the only drug capable of eliminating dormant parasites residing in the host's liver; unlike the previous quinoline-based drugs, primaquine **4** has rarely reported adverse psychiatric effects and is generally tolerable [11]. However, resistance to primaquine **4** has also been observed [12]

Pyrimethamine **8** was first introduced as a single drug, however, within a short period of time, resistance to *P. falciparum* and *P. vivax* was observed. Therefore, this drug was paired with another antifolate drug named sulfadoxine **7** which proved effective against chloroquine-resistant malaria, but again, resistance rapidly reappeared [9]. Chemical structures of both agents can be viewed in **Figure 3**.

More recently, lumefantrine **9** pyronaridine **10** (**Figure 4**) and some of the antimalarial agents mentioned previously have been partnered up with the indispensable artemisinin drugs (**Figure 5**) to create artemisinin-based combination therapies (ACT), see **Table 2**.

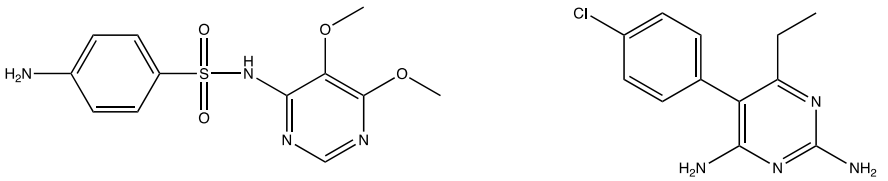
Artemisinins are a valuable set of tools in the battle against malaria. They have a quick onset of action and can be administered intravenously to treat individuals with severe malaria. The use of artemisinin drugs outside of ACT is highly discouraged by the WHO due to the fear of resistance. However, reports of resistance have been reported in South-east Asia [9]. The first reported case of ACT-resistance occurred 10 years ago in Cambodia and since then ACT-resistance is now predicted to cause over 116,000 deaths globally per annum and cost over $32 million per year to treat [13].

The atovaquone **14**/proguanil **15** (**Figure 6**) combination (Malarone®), is an effective but expensive prophylactic therapy which also has reports of resistance [9].



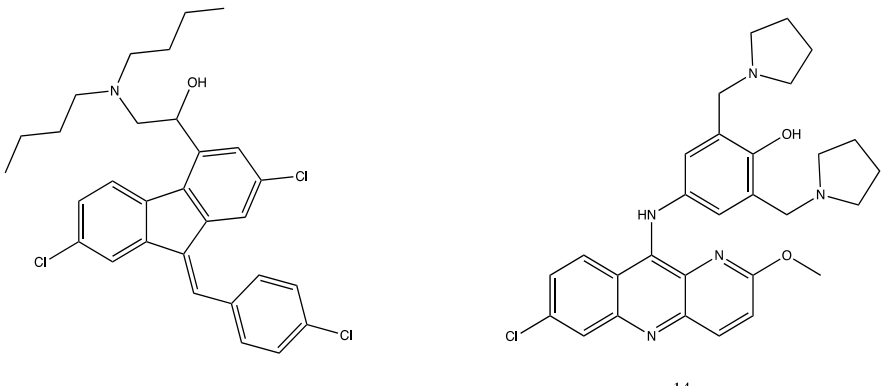
4 5 6

Figure 2. Chemical structures of Primaquine **4** quinine **5** and mefloquine **6**.



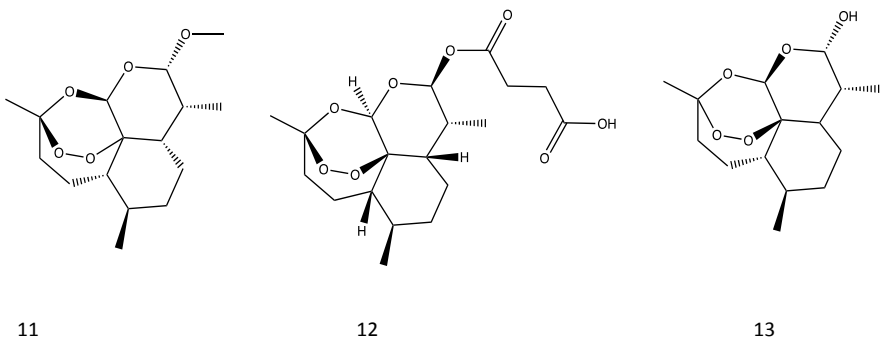
7 8

Figure 3. Chemical structures of Sulfadoxine **7** and Pyrimethamine **8**.



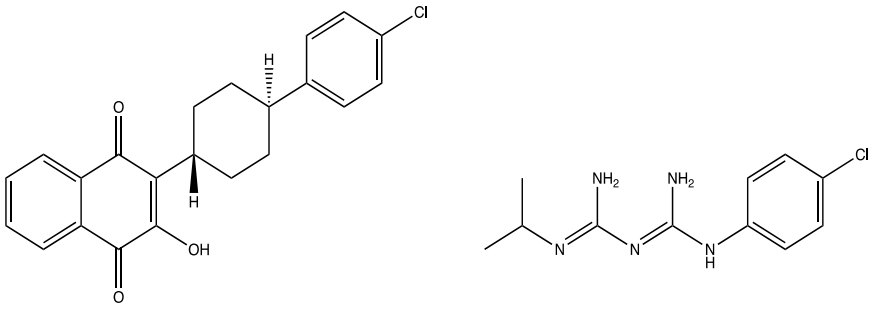
9 10

Figure 4. Chemical structures of Lumefantrine **9** and Pyronaridine **10**.



11 12 13

Figure 5. Chemical structures of Artemether **11**; Artesunate **12** and Dihydroartemisinin **13**.



14 15

Figure 6. Chemical structures of Atovaquone **14** and Proguanil **15**.

Table 2. Artemisinin-based combination therapy (ACT) recommended by WHO.

Artemether and Lumefantrine
Artesunate and Amodiaquine
Artesunate and Mefloquine
Dihydroartemisinin and Piperaquine
Artesunate and Pyronaridine
Artesunate and Sulfadoxine–Pyrimethamine

1.4. Malarial Resistance

Almost all antimalarial drugs have reported resistance, which results in a delayed or insufficient clearance of *Plasmodium* in the host's blood; this may cause profound consequences upon the host's wellbeing and increases the chances of transmission to another host [14].

Despite the many attempts by humans to eradicate malaria, *Plasmodium* has always had the ability to mutate and navigate around the actions of antimalarial drugs. The urgency to combat resistance has increased as almost all the antimalarial drugs have recorded cases of resistance which has denounced the once indispensable agents. The race between the development of novel antimalarial drugs and the uprising of resistance has intensified, therefore, identifying new targets and developing new molecules that can interact with these targets is urgently required in order to produce novel antimalarial drugs [15].

1.5. Molecular Docking

During the last three decades, computational methods have played a major role in drug discovery and design; in particular structure-based methods such as molecular docking has become an important technique [16] [17].

Molecular docking studies have been routinely used to better understand current antimalarial drugs on the market. A study in 2013 found that quinoline-based drugs have better binding modes as non-competitive inhibiters than as competitive inhibiters to *P. falciparum* lactate dehydrogenase, a vital enzyme the parasite requires for energy [18]. This method has also paved the way for

lead compounds to be considered for pharmaceutical research. A study in India found a lead compound with positive docking and pharmacophores features for leucine amino peptidase, an enzyme vital for the growth and development of *P. vivax* [19].

AutoDockVina is a high speed, popular molecular docking programme which is compatible with UCSF Chimera, a molecular visualization software known for its numerous functions and capabilities [20] [21]. Both platforms will be at the heart of this study.

1.6. Target Protein

P. falciparum has been studied intensively due to its high mortality rate, however, *P. vivax* is more widely distributed around the world and brings a larger social economic burden, therefore, searching for an effective treatment against this parasite is also important [4].

N-myristoyltransferase (NMT) is an essential enzyme found in *P. vivax* and humans, which catalyses the bonding of a saturated 14-carbon fatty acid named myristate to the N-terminal glycine of target proteins; this process is called myristoylation [22]. A number of essential proteins such as protein kinase 1 and glideosome found in malarial parasites need to undergo myristoylation to carry out their biological functions, therefore, by inhibiting NMT, the parasite should fail to thrive [23]. Variations between parasitic and human NMT makes it a highly promising selective target [24].

Several published studies have aimed to discover potential inhibitors of *Plasmodium vivax* (Pv) NMT. A United Kingdom study in 2012 successfully discovered a hit compound named 3-butyl-4-((2-cyanoethyl)thio)-6-methoxy-2-methyl-quinoline using a high throughput screening technique involving a fluorogenic assay [24]. A similar study in the same year tested benzothiophene containing inhibitors using structure activity relationship techniques and discovered a lead compound for further development [23].

There are several published studies involving PfNMT, however, the current lack of molecular docking studies involving *Pv*NMT has encouraged this study to pursue this as the target protein of interest. The 3D structure of *Pv*NMT (**Figure 7**).

The overall aim of this study was to prepare and evaluate a series of naphthoquinone and aryl diketone ligands for potential use as *Pv*NMT inhibitors using computational docking techniques.

2. Materials and Methods

The chemical diaries of Napthoquinones and diketones were used for the purpose of this screening, because the Napthoquinones and diketones have pharmacophores that worthy of further investigations. Naphthoquinones have been found to be active towards some cancer, and diketones are very good

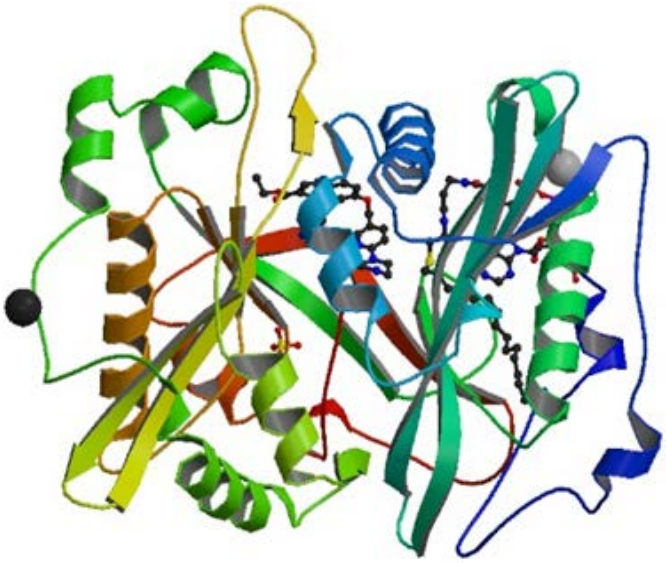


Figure 7. 3D structure of *Plasmodium vivax* N-myristoyltransferase, PDB ID: 5G22 [25].

anti-microbial activities. The structures of Napthoquinones and diketones used for this docking investigation were obtained from the NCBI Pub-Chem database.

2.1. Target Protein and Ligand Selection

A range of ligands will be docked against PvNMT to yield an array of results. Firstly, naphthoquinone compounds will be docked to the target protein as they have been known to possess antimalarial activity [26]. There is an antimalarial drug currently on the market called atovaquone that belongs to the class of naphthoquinones, therefore, these compounds are a promising choice as ligands. Aromatic diketones, cromolyn and FDA approved antimalarial drugs will also be docked with PvNMT for comparison purposes.

The three-dimensional structure of *P. vivax* N-myristoyltransferase was downloaded as a PDB file from the RCSB Protein Data Bank (PDB ID: 5G22). This protein was opened in Auto Dock Tools version 1.5.6 where the co-ordinates of the binding pocket were determined using the grid box function. The figures used are shown below in **Table 3**.

The PDB file of the protein was opened in UCSF Chimera version 1.12 and all non-standard atoms and bonds were removed. Using the dock prep function, water molecules were deleted, partial charges were assigned and hydrogens were added to the protein. The protein was then saved in the Mol 2 format ready for the docking stage.

2.2. Ligand Preparation

The naphthoquinones and aryl diketones compounds on PubChem were long-listed for the docking stage. The canonical SMILES of the compounds were

Table 3. Co-ordinates of the binding pocket for the target protein.

	Size	Centre
X dimension	20	17
Y dimension	28	12
Z dimension	30	62

entered into UCSF Chimera using the build structure tool. The compound was then prepared by adding hydrogens using the structure editing function and moved the system towards its minimized energy using the minimize structure tool. The ligand was named "target molecule #" and saved in the Mol2 format ready for the docking stage.

2.3. Molecular Docking

On a new UCSF Chimera session, firstly, the Mol2 file of the protein was opened followed by the target moleculea. Using the structure/binding analysis tool, the AutoDockvina function was opened. The PDB format of the protein was selected as the receptor and the SMILES of the target molecule was selected as the ligand. An output file was created and the co-ordinates of the binding pocket, as shown in Table 3 was entered. All receptor and ligand options were set as true and the exhaustiveness of search was set at the maximum of 8. Also, the number of binding modes was selected as 9. Vina.exe via a local path was chosen as the executable location and docking was applied. All naphthoquinone and aryl diketone compounds were docked with *P. vivax* N-myristoyltransferase by following this method.

2.4. Docking Analysis

Analysis was carried out for all docking sessions on UCSF Chimera. All intra-residue hydrogen bonds were made visible by altering the hydrogen bond function on Chimera. The line width was kept at the default 4.0, however, the number of angstroms was altered to 3.0. Applying these changes displayed all hydrogen bonds (less than approximately 5 angstroms) between the protein and the 9 binding modes of the ligand. Each of the nine binding modes for all 166 ligands were analysed for any hydrogen bond(s) less than 3 angstroms. The amino acid residue involved in the hydrogen bond, its position and the number of angstroms of the bond were all recorded during the docking analysis phase.

3. Data Analysis

3.1. Binding Mode Analysis

Binding mode analysis were carried out for all docking sessions by relaunching the python files on UCSF Chimera. All intra-residue hydrogen bonds were made visible by altering the hydrogen bond function on Chimera. The line width was kept at the default 4.0, however, the number of angstroms was altered to 3.0.

Applying these changes displayed all hydrogen bonds (less than approximately 5 angstroms) between the target protein and ligand across the 9 binding modes.

Angstroms is a unit of length equivalent to 0.1 nanometres and as the shorter the length of a hydrogen bond results in a stronger interaction, finding bonds with short angstroms will identify binding modes with good affinity [27]. By hovering over a hydrogen bond, the number of angstroms as well as the name and position of the amino acid involved in the bond were found (**Figure 8**). All hydrogen bonds across the 9 binding modes for all 166 ligands were analysed for any hydrogen bond(s) less than 3 angstroms. The amino acid residue involved in these hydrogen bonds, its position and the number of angstroms of the bond were recorded.

These data was collated and evaluated on Microsoft Excel and the short-listing process was undertaken to identify hit compounds.

3.2. Docking Scores Analysis

As well as generating binding modes, AutoDockVina also uses a combination of knowledge-based and empirical scoring functions to predict the binding affinities of the ligands to the target protein; this is represented as the docking score [28]. These figures were obtained for the short-listed hit compounds from the View Dock window on UCSF Chimera (**Figure 9**). These data was collated and evaluated.

Christopher Lipinski formulated a tool named the rule of five (Ro5) to evaluate the drug-likeness of chemical compounds; he based this rule on the basis that most orally delivered drugs are small and moderately lipophilic molecules [29]. By obtaining the Ro5 parameters, the drug-likeness of the hit compounds was studied. This involved acquiring molecular weights from PubChem and cLogP values from an online software called ALOGPS 2.1. Also, the hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA) for each binding mode displaying hydrogens under 3 angstroms were recorded from ViewDock.

Twelve FDA approved antimalarial drugs were also docked with *Pv*NMT by following the same method used to dock the 166 ligands (Section 2.3). The hydrogen bond interactions and docking scores data were also obtained, recorded and evaluated by following the same methods (Sections 3.1 and 3.2). This data was used for comparative purposes.

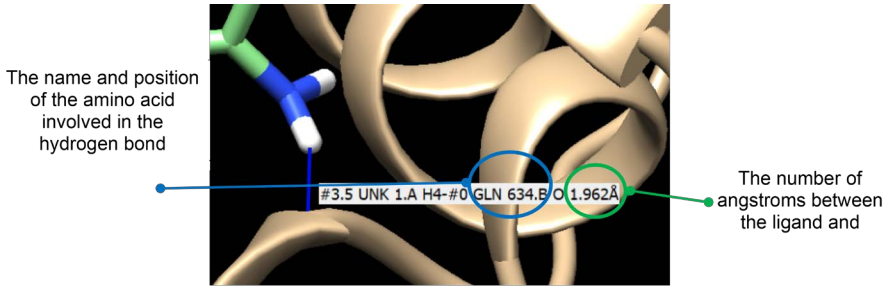


Figure 8. Visualisation of the information gathered from the hydrogen bonds.

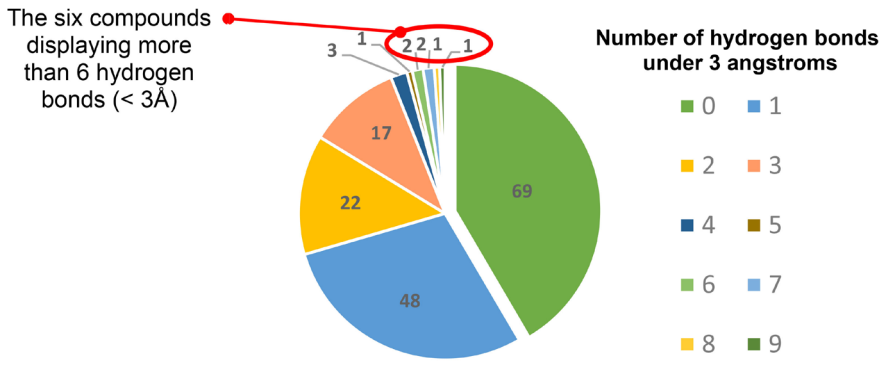


Figure 9. Number of target molecules displaying 0 - 9 hydrogen bonds (<3Å) across all binding modes with *Pv*NMTData acquired via binding mode analysis on AutoDockVina, *see Section* 3.1.

4. Results

4.1. Potential Hit Compounds Identification

All 166 target molecules, (labelled TM1-100 for naphthoquinones and TM101-166 for aryl diketones) were successfully docked to *Pv*NMT. Analysis of the binding modes revealed a range in the number of hydrogen bonds below 3 angstroms (Å) between the set. **Figure 10** displays this data and reveals 69 of the 166 target molecules (42%) displayed a lack of hydrogen bonds under 3Å. Therefore, the deduction can be made that these ligands have a lack of strong interactions with *Pv*NMT.

The remaining 97 target molecules (58%) displayed at least one hydrogen bond (<3Å) across the 9 binding modes. As the number of the hydrogen bonds (<3Å) increases, the strength of the interactions also increases. Therefore, ligands with the highest number of hydrogen bonds (<3Å) were short-listed as potential hit compounds.

Figure 9 shows that the number of target molecules decreases with increasing hydrogen bonds (<3Å). This facilitated the short-listing process. Out of the 166 target molecules, six displayed more than 6 hydrogen bonds (<3Å) with *Pv*NMT and were shortlisted as the top six hit compounds. The chemical structures of the hit compounds are shown in **Figure 10**.

4.2. Analysis of the Binding Modes

4.2.1. Potential Hit Compound Analysis

Further analysis of the binding modes were undertaken and the number of hydrogen bonds (<3Å) for the individual binding modes as well as the name and position of the amino acid residue involved in the hydrogen bond was obtained and recorded (**Table 4**). A total of 44 hydrogen bonds (<3Å) were observed across the six hit compounds.

The range of the amino acid residues involved in these observed hydrogen bonds are shown in **Figure 11**. A total of 10 different amino acids at 17 different position were involved. Glutamic acid (789.C), serine (245.A) and threonine (315.A) shared a total of 28 out of the 44 hydrogen bonds (64%), which marks

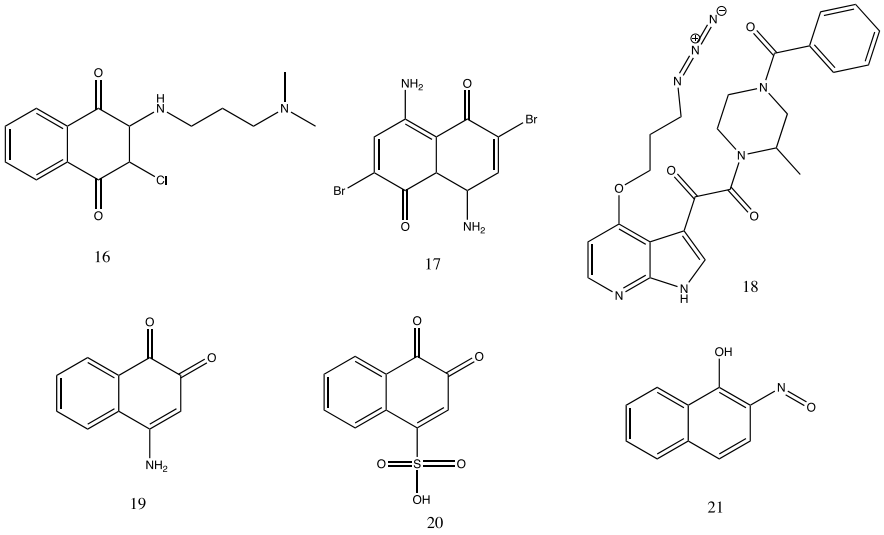


Figure 10. Chemical structures of the 6 hit compounds, TM94 **16**, TM6 6**17**, TM165 **18**; TM40 **19**; TM65**20**; and TM81 **21**.

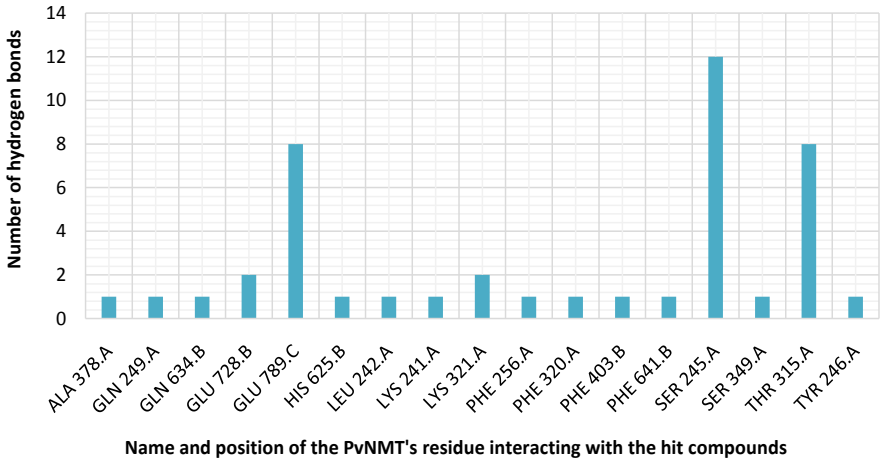


Figure 11. The number of potential hit compound hydrogen bonds (<3Å) with *Pv*NMTs residue.

these residue positions as hydrogen bonding hot spots. This data was used to research whether these hit compounds were binding to a recognised essential amino acid.

As of late 2017, there is a lack of data regarding the specific roles of these amino acid residues within *Pv*NMT. If glutamic acid (789.C), serine (245.A) and threonine (315.A) were found to be essential amino acids which the enzyme requires to function, therefore, the hit compounds could be selectively targeting an ideal location.

By comparing the potential hit compounds between themselves, TM165 displayed the highest total number of hydrogen (9) bonds (<3Å) across the 9 binding modes. However, TM40 displayed the highest total number hydrogen (3) bonds (<3Å) in one binding mode (pose 7) with an additional two bonds over 3

Table 4. The number of hydrogen bonds (<3Å) for the six potential hit compounds including the name and position of the interacting enzyme residue.

Target molecule number (TM#)	IUPAC Name	Binding mode number	Total number of hydrogen bonds below 3 angstroms	Number of hydrogen bonds (<3Å) at each pose	Name and position of the enzyme's residue interacting with the target molecule including (angstroms, Å)
TM40	4-aminonaphthalene-1, 2-dione	4	8	1	GLU 728.B (2.158)
		6		2	PHE 403.B (2.955) + LYS 321.A (1.877)
		7		3	SER 349.A (2.410) + LYS 321.A (1.867) + PHE 320.A (2.335)
		8		1	GLU 728.B (2.279)
		9		1	ALA 378.A (2.324)
TM65	3,4-dioxonaphthalene-1-sulfonic acid	1	6	1	THR 315.A (2.909)
		4		2	GLU 789.C (2.415) + GLU 789.C (2.622)
		5		2	GLN 249.A (2.891) + TYR 246.A (2.544)
		7		1	THR 315.A (2.970)
TM66	4,8-diamino-2,6-dibromonaphthalene-1,5-dione	1	8	1	SER 245.A (2.695)
		3		1	SER 245.A (2.588)
		4		2	SER 245.A (2.590) + THR 315.A (2.158)
		5		1	GLN 634.B (1.962)
		6		1	SER 245.A (1.911)
		7		1	SER 245.A (2.028)
		9		1	LYS 241.A (2.607)
TM81	2-nitrosonaphthalen-1-ol	1	6	1	GLU 789.C (2.179)
		2		2	PHE 641.B (2.120) + HIS 625.B (2.379)
		5		1	PHE 256.A (2.117)
		7		1	GLU 789.C (2.323)
		8		1	THR 315.A (2.335)
TM94	2-chloro-3-[3-(dimethylamino) propylamino] naphthalene-1,4-dione	1	7	1	GLU 789.C (2.442)
		5		1	SER 245.A (2.180)
		6		1	SER 245.A (2.093)
		7		2	SER 245.A (2.210) + GLU 789.C (2.313)
		8		1	SER 245.A (2.394)
		9		1	SER 245.A (2.236)
TM165	1-[4-(3-azidopropoxy)-1 H-pyrrolo[2,3-b]pyridin-3-yl]-2-[(2R)-4-benzoyl-2-methylpiperazin-1-yl]ethane-1,2-dione	1	9	2	THR 315.A (2.885) + THR 315.A (2.304)
		2		1	GLU 789.C (2.453)
		3		2	GLU 789.C (2.789) + SER 245.A (1.953)
		4		1	THR 315.A (2.269)
		6		1	THR 315.A (2.160)
		8		1	SER 245.A (2.124)
		9		1	LEU 242.A (2.000)

angstroms (4.335Å and 5.436Å). This binding mode also recorded the hydrogen bond with the lowest angstrom (1.867Å) compared to all the 44 observed hydrogen bonds.

Figures 12-17 display the binding mode for each potential hit compounds which displayed the highest number of hydrogen bonds (<3Å) with the lowest angstroms.

4.2.2. Comparison with FDA Approved Antimalarial

As explained in section 3.2, 12 antimalarial drugs were docked and analysed following the same methods used for the ligands. Out of the 12 drugs, eleven displayed a lower number of hydrogen bonds (<3Å) across the 9 binding modes compared to the six potential hit compounds, as shown in Table 5. The hit compounds displayed 6 or more hydrogen bonds (<3Å), whereas the highest of these eleven drugs displayed only 4 (primaquine and pyrimethamine).

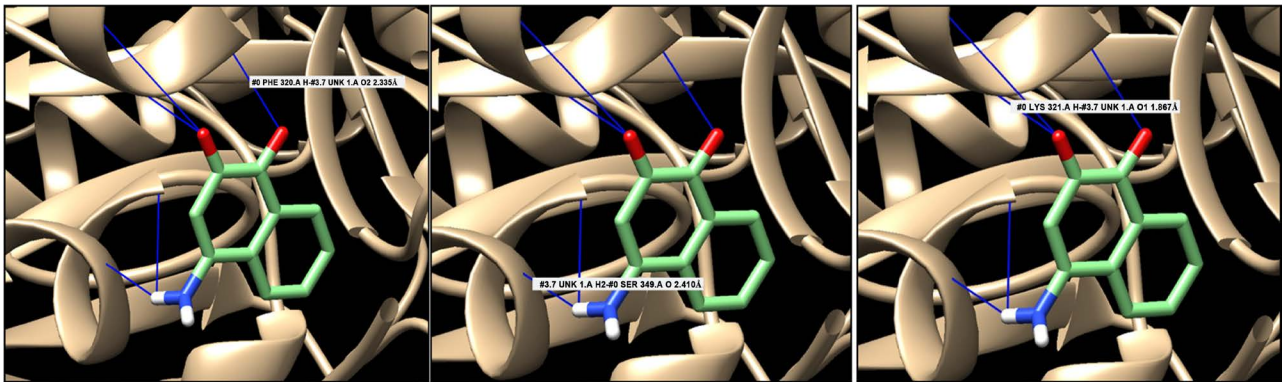


Figure 12. The hydrogen bonds (<3Å) for TM40, pose 7.

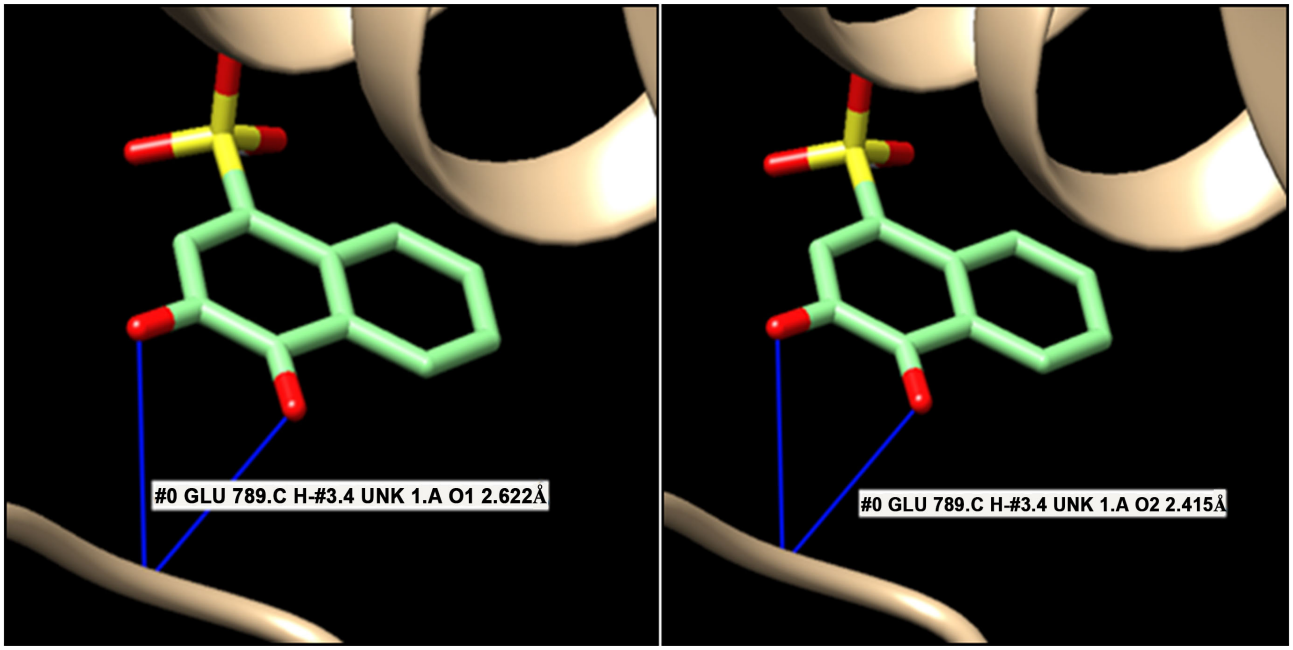


Figure 13. The hydrogen bonds (<3Å) for TM65, pose 4.

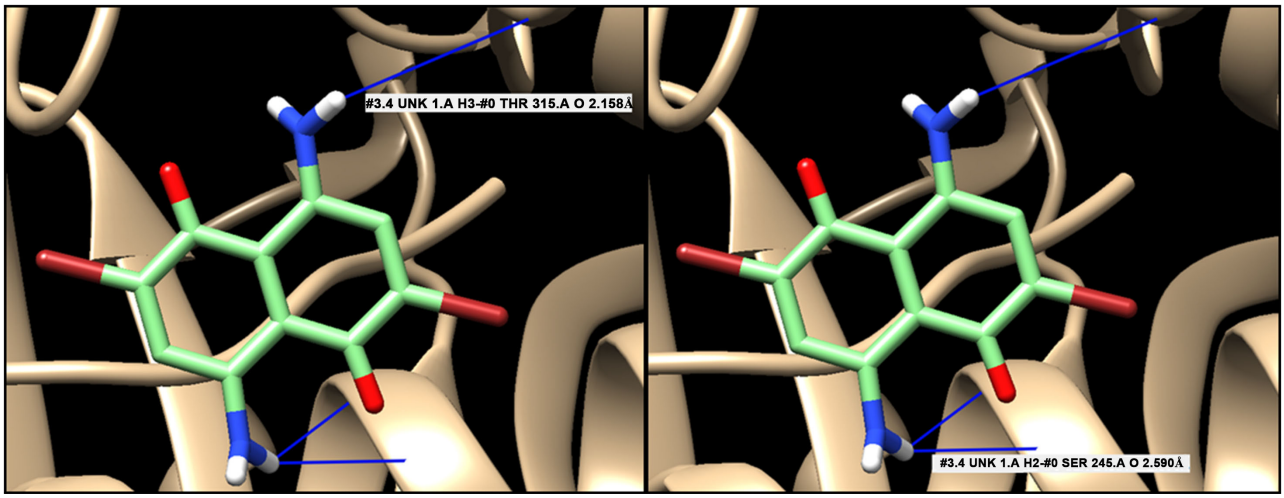


Figure 14. The hydrogen bonds (<3Å) for TM66, pose 4.

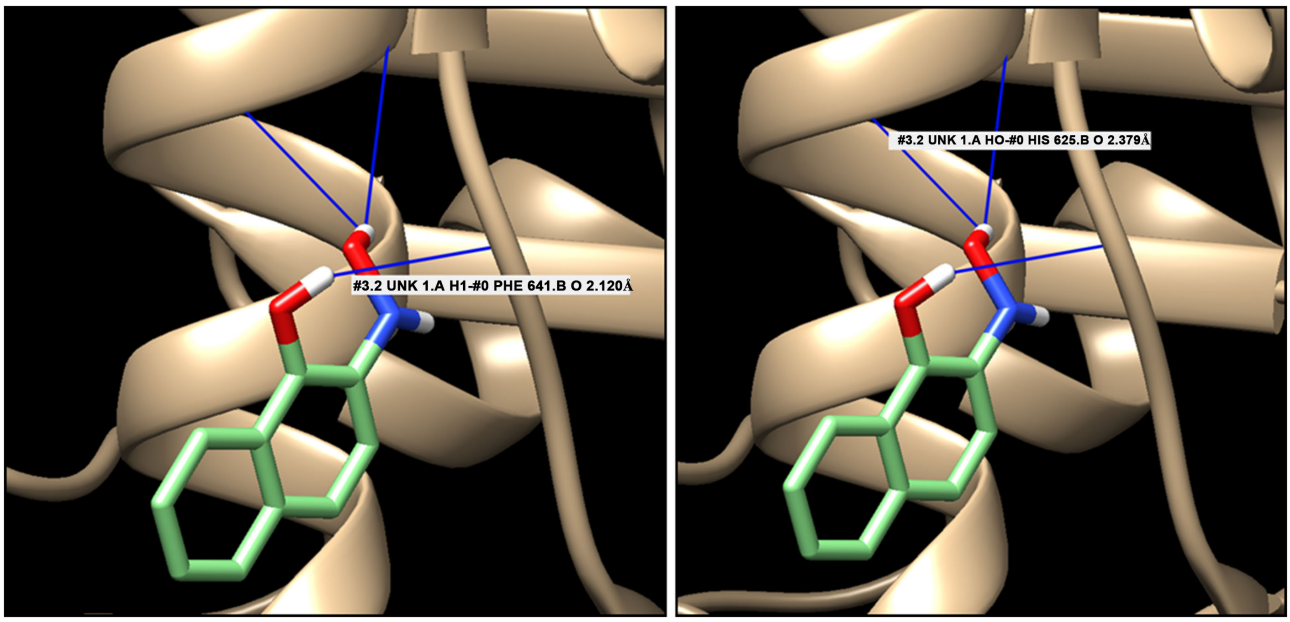


Figure 15. The hydrogen bonds (<3Å) for TM81, pose 2.

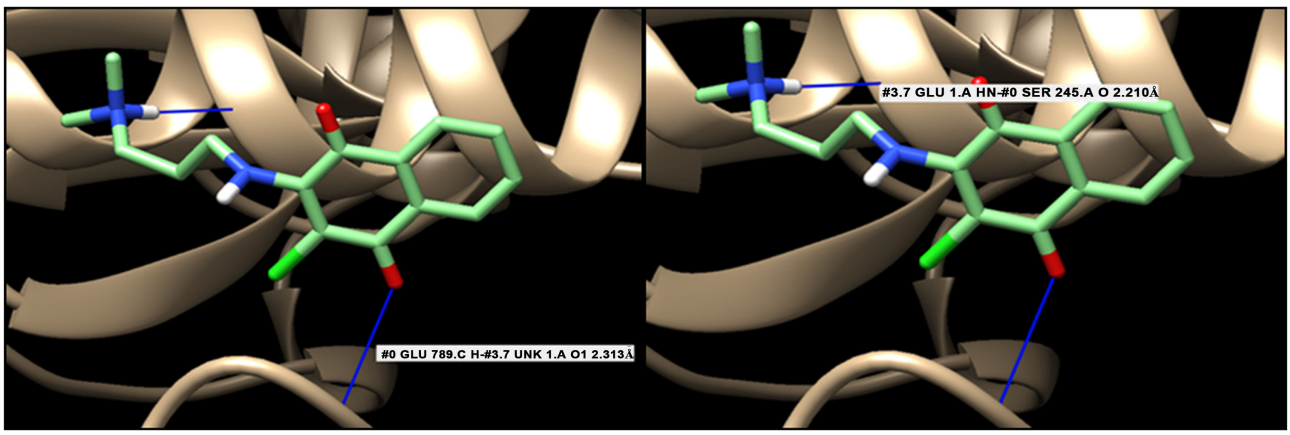


Figure 16. The hydrogen bonds (<3Å) for TM94, pose 7.

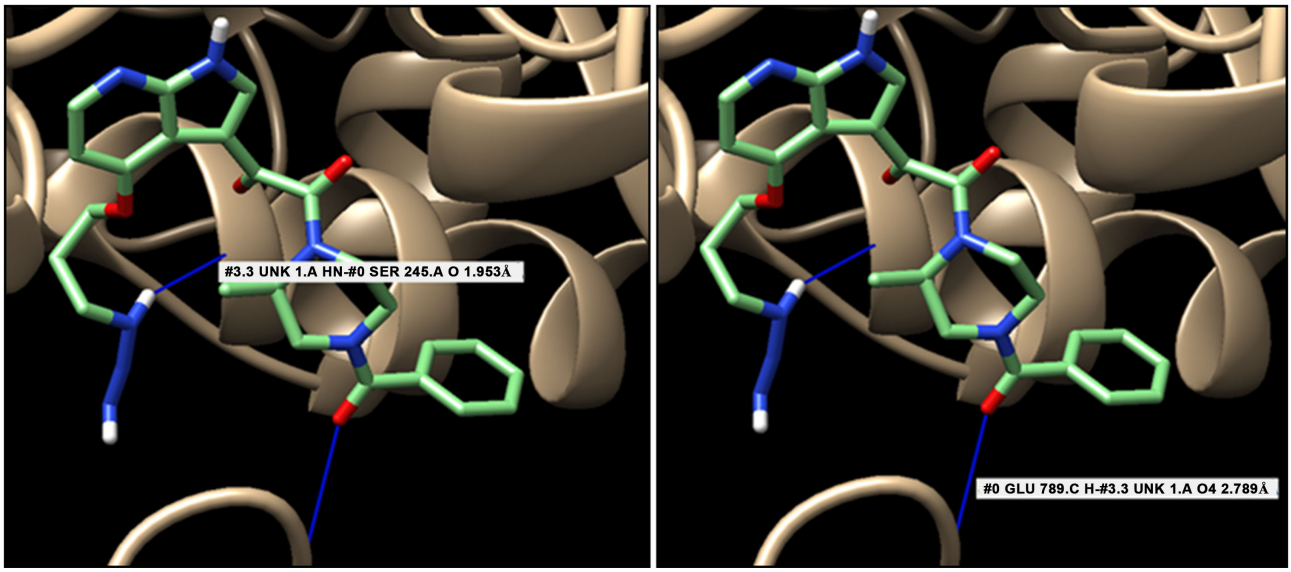


Figure 17. The hydrogen bonds (<3Å) for TM165, pose 3.

However, proguanil outperformed not only the other 11 drugs but also the 6 potential hit compounds by displaying 10 hydrogen bonds (<3Å) across the 9 binding modes. The fourth binding mode for proguanil displayed three of these bonds and are shown in **Figure 18**.

The varying binding locations between the potential hit compounds and proguanil must be noted. As explained in the previous section, the hit compounds predominately interacted with glutamic acid (789.C), serine (245.A) and threonine (315.A), whereas progunail does not. The antifolate drug instead mainly interacted with lysine (241.A) and leucine (242.A).

4.2.3. Docking Scores Analysis

The docking scores for the binding modes of the shortlisted compounds and the 12 FDA approved drugs which displayed hydrogen bonds (<3Å) are listed in **Table 6**. The scores are listed in ascending order as the lower docking scores are generated by the more active ligands [30]. All seven of TM165's observed binding modes had the best docking scores indicating that this ligand was calculated to have the best binding affinity out of all the hit compounds.

The poses which displayed the highest number of hydrogen bonds (<3Å) with the lowest angstroms did not correlate with the docking scores. Pose 7 of TM94 displayed two hydrogen bonds (<3Å) which is double of any other pose, however, TM94_7 generated the lowest docking score. Similarly, proguanil generated a docking score in the lower half of the docking score range.

In determining the docking for the poses, ideally the poses with RMSD value of zero for both lower and upper bounds should be deemed to have highest binding affinity, but, it must be noted that the docking scoring function has conventionally been associated with poor accuracy, which renders it one of the most important limitation of molecular docking [28]. This may explain the poor correlation.

Table 5. Number of hydrogen bonds (< 3Å) between FDA approved drugs and *Pv*NMT

Drug class	Drug name	Number of hydrogen bonds (<3Å)	Conformation	Name and position of the enzyme's residue interacting with the target molecule including (angstroms, Å)
4-aminoquinoline	Chloroquine	1	7	GLU 789.C (2.908)
	Amodiaquine	2	2	LEU 242.A (2.985)
			6	LEU 242.A (2.095)
8-aminoquinoline	Primaquine	4	3	TYR 246.A (2.753)
			4	SER 245.A (2.246)
			5	TYR 246.A (2.962) + SER 245.A (2.429)
Arylamino alcohol	Mefloquine	3	1	LEU 242.A (2.428)
			4	THR 315.A (2.129)
			7	SER 245.A (2.643)
Sesquiterpene lactone endoperoxides	Artemether	0		
	Artesunate	2	5	TYR 246.A (2.574) + SER 245.A (2.139)
	Dihydroartemisinin	0		
Antifolate	Pyrimethamine	4	3	SER 245.A (2.012)
			4	SER 245.A (2.402)
			8	SER 245.A (2.703)
			9	THR 315.A (2.228)
	Sulfadoxine	3	1	ILE 283.A (2.214)
			2	ALA 639.B (2.559)
			9	LEU 242.A (2.239)
	Proguanil	10	1	LYS 241.A (2.363) + LEU 242.A (2.032)
			2	LEU 242.A (2.570)
			4	LYS 241.A (2.522) + LEU 242.A (2.834) + LEU 242.A (2.021)
			5	ALA 763.B (2.241) + GLN 634.B (2.578)
			8	GLN 249.A (2.230) + ALA 378.A (2.937)
Naphthoquinone	Atovaquone	0		
Antibiotic	Doxycycline	3	4	GLN 634.B (2.147)
			7	SER 245.A (2.506)
			9	THR 315.A (2.577)

4.2.4. Drug-Likeness of the Hit Compounds

The four components that form Lipinski's Ro5 is shown in Figure 19.

This rule was applied to the six shortlisted compounds and Table 7 shows that all potential hit compounds obeyed the Ro5 with no violations. This indicates that these compounds possess favourable characteristics to become orally active drugs.

The molecular mass of TM165 was on the upper end of the limit with a molecular weight of 475.5 g/mol (1 g/mol ≅ 1 Dalton), which is 25 Daltons from a

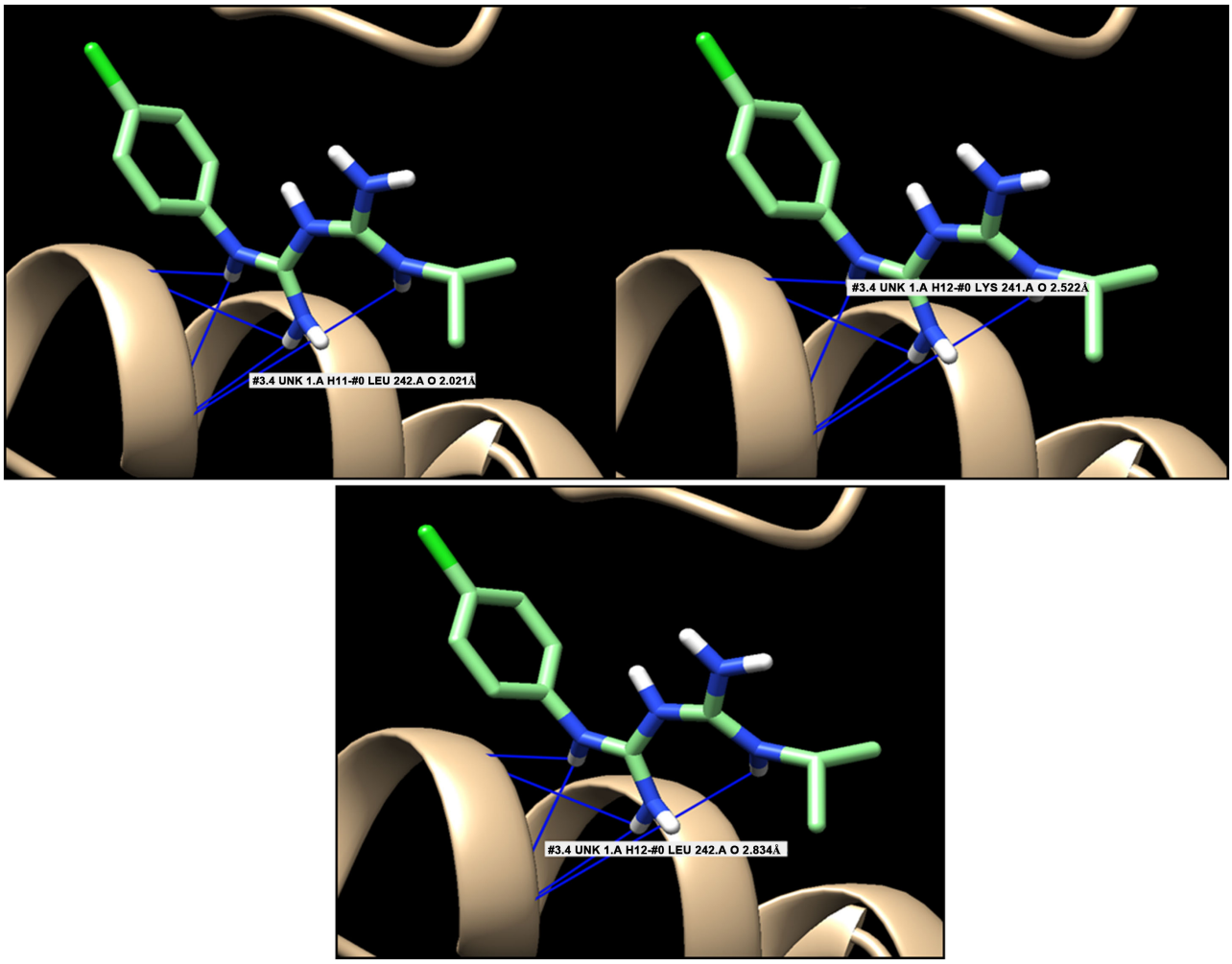


Figure 18. The hydrogen bonds (<3Å) for proguanil, pose 4.

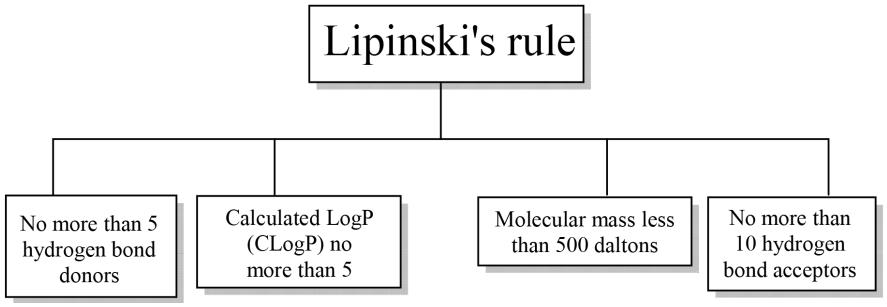


Figure 19. The components of Lipinski's rule [29].

violation. Exceeding the parameters may have a negative impact upon absorption and permeation compared to the other hit compounds [29]. The other parameters for all hit compounds were well within the Ro5 limits.

This section has presented evidence for six potential hit compounds as promising candidates as potential *Pv*NMT inhibitors. The rationale behind shortlisting these candidates from 166 target molecules was explored. This was followed by displaying the compounds' superior hydrogen bonding compared to the

Table 6. The docking scores for the poses of the six shortlisted compounds and docked antimalarial drugs with hydrogen bonds < 3Å in ascending order.

Target molecule_ (pose number)	Docking score
TM165_1	−7.4
TM165_2	−7.3
Mefloquine_1	−7.3
TM165_3	−7.2
Doxycycline_4	−7.2
TM165_4	−7.0
TM165_6	−6.9
TM165_8	−6.8
TM165_9	−6.8
Doxycycline_7	−6.6
Mefloquine_4	−6.5
Artesunate_5	−6.5
Doxycycline_9	−6.5
Amodiaquine_2	−6.4
Mefloquine_7	−6.3
TM65_1	−5.9
Amodiaquine_6	−5.8
Proguanil_1	−5.8
Pyrimethamine_3	−5.7
Proguanil_2	−5.7
TM40_4	−5.5
TM40_6	−5.5
TM40_7	−5.5
TM66_1	−5.5
TM66_3	−5.5
TM81_1	−5.5
Sulfadoxine_1	−5.5
TM40_8	−5.4
TM66_4	−5.4
TM81_2	−5.4
Sulfadoxine_2	−5.4
Proguanil_4	−5.4
Proguanil_5	−5.4
TM40_9	−5.3
TM65_4	−5.3
Primaquine_3	−5.3
Primaquine_4	−5.3
Primaquine_5	−5.3

Continued

TM65_5	−5.2
TM65_7	−5.2
TM81_5	−5.2
TM94_1	−5.2
Chloroquine_7	−5.2
Pyrimethamine_4	−5.2
Proguanil_8	−5.2
Sulfadoxine_9	−5.1
TM66_5	−5.0
TM81_7	−5.0
TM94_5	−5.0
TM66_6	−4.9
TM66_7	−4.9
TM66_9	−4.9
TM94_6	−4.9
TM81_8	−4.8
TM94_7	−4.8
TM94_8	−4.8
TM94_9	−4.8
Pyrimethamine_8	−4.8
Pyrimethamine_9	−4.0

majority of the docked FDA approved drugs. Furthermore, the drug-likeness of all potential hit compounds was confirmed by using the Lipinski's Ro5.

4.3. Strengths and Limitations

The strengths and limitations of this study lies with the largest variable of this study, the molecular docking technique. AutoDockVina was the chosen docking software in this study, however, there are more than 60 developed docking tools available; some examples include DOCK, MOE, AutoDock and GOLD (Pagadala, Syed and Tuszynski, 2017). AutoDockVina was released in 2010 as a successor to AutoDock; the upgrade was promoted to possess twice the speed and an improved binding mode accuracy compared to its predecessor [21].

All docking softwares including AutoDockVina are subject to the universal limitations of molecular docking. A major weakness is the accuracy of the scoring function which is one of the most important component of this computational technique. Various software's utilise different scoring function techniques such as empirical, force-field and knowledge-based, which all carry limitations. However, several comparative studies have promoted the accuracy of AutoDockVina. A recent comparative study of docking softwares ranked AutoDockVina along with GOLD and MOE as the best tools for accurate prediction of ranking poses with the best docking scores [31].

Table 7. Analysis of the six potential hit compounds against Lipinski's rule of 5 parameters.

Target molecule number (TM#)	IUPAC name	Molecular weight (g/mol)	Number of hydrogen bond donors		Number of hydrogen bond acceptors		CLogP range
TM40	4-aminonaphthalene-1,2-dione	173.171	Pose 4	1	Pose 4	1	1.55 - 2.43
			Pose 6	3	Pose 6	3	
			Pose 7	3	Pose 7	3	
			Pose 8	2	Pose 8	2	
			Pose 9	1	Pose 9	1	
TM65	3,4-dioxonaphthalene-1-sulfonic acid	238.213	Pose 1	2	Pose 1	2	−0.55 - 2.77
			Pose 4	2	Pose 4	1	
			Pose 5	2	Pose 5	2	
			Pose 7	2	Pose 7	3	
TM66	4,8-diamino-2,6-dibromonaphthalene-1,5-dione	345.978	Pose 1	1	Pose 1	1	1.28 - 3.51
			Pose 3	1	Pose 3	1	
			Pose 4	2	Pose 4	2	
			Pose 5	1	Pose 5	1	
			Pose 6	1	Pose 6	1	
			Pose 7	2	Pose 7	2	
			Pose 9	1	Pose 9	1	
TM81	2-nitrosonaphthalen-1-ol	173.171	Pose 1	1	Pose 1	1	2.43 - 2.70
			Pose 2	2	Pose 2	2	
			Pose 5	1	Pose 5	1	
			Pose 7	2	Pose 7	2	
			Pose 8	2	Pose 8	2	
TM94	2-chloro-3-[3-(dimethylamino)propylamino]naphthalene-1,4-dione	292.763	Pose 1	1	Pose 1	1	2.36 - 3.56
			Pose 5	1	Pose 5	1	
			Pose 6	1	Pose 6	1	
			Pose 7	2	Pose 7	2	
			Pose 8	1	Pose 8	1	
			Pose 9	1	Pose 9	1	
TM165	1-[4-(3-azidopropoxy)-1H-pyrrolo[2,3-b]pyridin-3-yl]-2-[(2R)-4-benzoyl-2-methylpiperazin-1-yl]ethane-1,2-dione	475.509	Pose 1	3	Pose 1	3	2.62 - 3.88
			Pose 2	2	Pose 2	2	
			Pose 3	4	Pose 3	5	
			Pose 4	1	Pose 4	1	
			Pose 6	2	Pose 6	3	
			Pose 8	3	Pose 8	3	
			Pose 9	3	Pose 9	3	

5. Conclusions

The six compounds presented similarities and differences to previously discovered inhibitors. Published hit compounds possessed a common pharmacophore of primary, secondary and tertiary amines [32]. This coincides with the majority of the six potential hit compounds generated from this study as five of the six compounds (TM40, TM66, TM81, TM94 and TM165) possess either primary, secondary or tertiary amines. This pattern is however not widely consistent as NMT inhibitors are known to display diverse binding modes which result in a range of different pharmacophores; this may explain the absence of these pharmacophores in TM65 [32].

The twelve FDA approved antimalarials were docked with *Pv*NMT even though they are not known to exert their action on this target protein. However, using currently marketed drugs as starting points in drug discovery provides a better practical and cheaper alternative compared to untested synthetic compounds [33]. The comparison of hydrogen bond interactions between the hit compounds and FDA approved drugs were analysed and the six shortlisted compounds were found to perform better than eleven of the twelve drugs. However, proguanil out performed all hit compounds and FDA approved drugs.

The varying binding locations between the potential hit compounds and proguanil must be noted. The potential hit compounds predominately interacted with glutamic acid (789.C), serine (245.A) and threonine (315.A), whereas progunailmainly interacted with lysine (241.A) and leucine (242.A). This finding shows that the six potential hit compounds displayed stronger hydrogen bonds at different locations compared to the 12 FDA approved antimalarial agents. This is important as drugs that exert their action at different locations of the target protein can lower the incidence of drug resistance. This forms the basis behind the originality of the potential hit compounds, and strengthens the case for using these compounds as starting points for development.

Furthermore, none of the shortlisted compounds violated any of the Lipinski's Ro5 parameters. This is a positive indication that these compounds not only show promising interactions, but also possess ideal characteristics to become desirable orally active therapeutic agents.

The potential hit compounds found in this study increases the number of potential inhibitors for *Pv*NMT As discussed in the previous section, these compounds can also aid further validation of *Pv*NMT as an ideal target protein by demonstrating selective inhibition which subsequently results in reductions in *Plasmodium* in human blood. Moreover, subject to further development, these compounds possess the potential to become hit compounds, which can be developed to Lead compounds and eventual antimalarial drugs. A novel therapeutic agent would be a huge leap forward in the race against antimalarial resistance.

In the event of these compounds demonstrating selective inhibition of *Pv* NMT with subsequent reductions in *Plasmodium* in the blood, then this protein will be further validated as a drug target.

Finally, even though there is no single docking program which has dominative benefits above others, as all are subject to the universal molecular docking limitations. However, the results generated from AutoDockVina has been proved to be a reliable choice compared to the other docking softwares and this give credibility to the six shortlisted compounds that we obtained during the course of this research. The aim of this study was met as six potential hit compounds were discovered.

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