In silico Analysis of Naphthoquinone Compounds as Potential Antimalarial Agents Targeting the Cytochrome bc_1 Complex

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INTRODUCTION

Atovaquone (Table 1) is used clinically to target *Plasmodium falciparum* bc_1 complex (Fig.1), which competitively inhibits ubiquinol oxidation, specifically inhibiting the mitochondrial electron transport chain at the cytochrome bc_1 complex. It is a hydroxyl-naphthoquinone analogue of ubiquinone that binds at the Q_o site of cyt bc_1 inhibiting oxidation of ubiquinol resulting in mitochondrial membrane potential collapse. But the parasite is rapidly developing resistance against it. Thus, there is an urgent need to develop new drugs with increased potency.

An extensive search of the literature identified 488 unique compounds, including 23 known inhibitors, targeting this site. Of which, 1,4-dihydronaphthalene-1,4-diones displayed potential activity & warranted further investigated

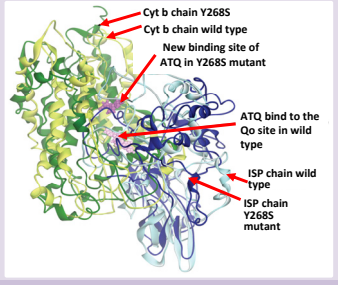

Figure 1: Binding site of atovaquone in *Plasmodium falciparum* cytochrome bc_1 complex.

AIMS

This study aimed to compare the selectivity, pharmacokinetic, antimalarial & toxicity profile of twenty-nine 1,4-naphthoquinones compounds to atovaquone; in an attempt to identify a potential lead molecule targeting *Plasmodium falciparum* cytochrome bc_1 complex.

METHODOLOGY

A database of small molecules was compiled from published research literature, including structural details of the compounds and antiplasmodial activities. To account for sensitivity of the parasite to atovaquone, the antimalarial activity ratio of pIC_{50} NPHQ to pIC_{50} ATQ was calculated.

Twenty-nine naphthoquinone (**NPHQ**) compounds were evaluated against cytochrome bc_1 complex (PDB 4PD4) to compare the docking scores with atovaquone. The 3D structures of all ligands were prepared using LigPrep program of Schrödinger Maestro with OPLS4 force field. The 3D structure for protein 4PD4 was subjected to protein preparation wizard of Schrödinger Maestro 12.7 version, where the protein was pre-processed and minimized. The co-crystallized ligand molecule (atovaquone) was used to identify the binding site and grid was generated using Glide Receptor Grid Generation program. This was followed by docking all ligands using xtra-precision mode to calculate glide score (docking score and G score).

The prime MM-GBSA v3.000 program was used to predict the relative binding-free energies and QikProp and Swiss-ADME programs were used to predict pharmacokinetic properties of the compounds in comparison to atovaquone.

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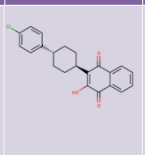
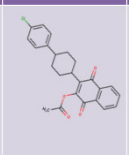
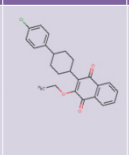
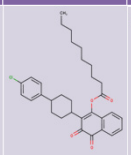
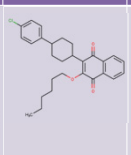
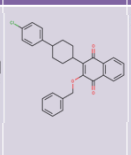
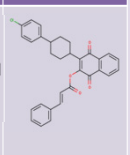
RESULTS AND DISCUSSION

Table 1: Selectivity, pharmacokinetic, antimalarial and toxicological profile of atovaquone & **NPHQ** compounds.

	Atovaquone	NPHQ-4	NPHQ-16	NPHQ-15	NPHQ-18	NPHQ-17	NPHQ-14
Chemical structures							
Docking scores (kcal/mol)	-11.221	-12.256	-11.703	-11.618	-11.372	-10.59	-8.777
Binding energies (kcal/mol)	-51.30	-57.23	-56.33	-65.95	-56.63	-68.84	-56.59
Antimalarial activity ratio	1	0.97	0.90	0.77	0.86	0.89	0.97
GIT absorption	High	High	High	Low	Low	Low	Low
BBB permeant	Yes	No	No	No	No	No	No
Inhibition of P450 cytochrome	CYP2C9 CYP2C19 CYP3A4 CYP1A2	CYP2C9 CYP2C19 CYP3A4	CYP2C9 CYP2C19 CYP3A4 CYP2D6	CYP2C9 CYP2C19 CYP3A4	CYP2C9 CYP2C19 CYP3A4 CYP2D6	CYP2C9 CYP2C19	CYP2C9 CYP2C19 CYP3A4

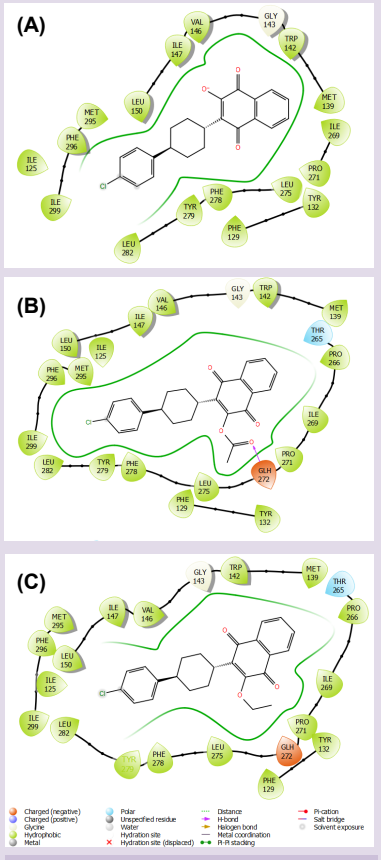


Figure 2: Ligand interaction diagram of Atovaquone (A) compared to **NPHQ-4** (B) & **NPHQ-16** (C).

- Analysis of the *in vitro* antimalarial activity of the compounds against a Nigerian *P. falciparum* strain, indicated inhibitory activity ranging from 1.45-13.5nM, with compound **NPHQ-15** >100nM; compared to atovaquone, 0.75nM. The antimalarial activity ratio as compared to atovaquone indicated that **NPHQ-4** & **NPHQ-14** were the most comparable to atovaquone (Table 1).
- The docking scores for the 29 naphthoquinone compounds ranged between -12.256 & -1.853kcal/mol, with **NPHQ-4**, **NPHQ-16**, **NPHQ-15**, **NPHQ-18** possessed docking scores in higher ranking order than atovaquone (Table 1; Figure 2).
- Prime MM-GBSA analysis yielded the relative binding energies (MMGBSA_dG_Bind) of **NPHQ-4**, **NPHQ-16**, **NPHQ-15**, **NPHQ-18** to be lower than atovaquone (Table 1), suggesting that these compounds bind more strongly to the target site than atovaquone.
- Atovaquone, **NPHQ-4** & **NPHQ-16** complied with all Lipinski Rule of 5 "drug-like" properties. Whilst **NPHQ-15** did not comply with 2 rules (MW >500, mlogP>4.15), contributing to a 94.5% predicted oral absorption compared to 100% for atovaquone & too polar to cross the blood brain barrier. **NPHQ-14**, **NPHQ-17, NPHQ-18** did not comply with 1 of Lipinski's rule of 5 (mlogP>4.15).
- By specifically substituting the hydroxyl group of atovaquone with acetoxy (**NPHQ-4**) and ethoxy (**NPHQ-16**) groups yielded more promising results, compared to the phenyl prop-2-enoate group of **NPHQ-14 or** benzyloxy group of **NPHQ-17**.
- Although **NPHQ-15** had a stronger binding affinity for the target site, the decanoate group at position 1 of **NPHQ-15** resulted in the loss of *in vitro* antimalarial activity, favourable pharmacokinetic & drug-likeliness properties required for a lead compound.
- The pharmacokinetic profiles for **NPHQ-4** & **NPHQ-16** suggest that the two compounds have high GIT absorption, but P450 cytochrome inhibition profile favours **NPHQ-4**.

CONCLUSIONS

Preliminary data indicated that by substituting the hydroxyl group of atovaquone with acetoxy (**NPHQ-4**) and ethoxy (**NPHQ-16**) groups, have the potential to serve as lead antimalarial compounds. Of these, **NPHQ-4** is favoured for designing inhibitors of the *Plasmodium* cytochrome bc_1 complex.

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