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Malaria

- 229 million recorded malarial infections annually (2019).
- Lethal cases have decreased by ~10% every five years since 2000 (2019).
- Malaria burden is highly uneven: Sub-Saharan Africa ~92% of lethal cases (2015)
- The R21 (modified RTS,S) vaccine displayed 77% efficacy young children (04/2021)
- The malaria-causing plasmodia can readily mutate, meaning there is no one 'silver-bullet'.
- ~80% of lethal malarial cases are related to *P. falciparum* infection.

Cytochrome bc_1 Complex and Atovaquone

- Cytochrome bc_1 is a dimeric multi-subunit, inner mitochondrial, membrane-bound protein
- Maintains electrostatic potential, driving ATP synthesis.
- Atovaquone is the only FDA-approved drug targeting the cyt bc1 complex of *P. falciparum*.
- Inhibitor of cyt bc1 at the Qo site
- Atovaquone/multi- drug-resistances emerging

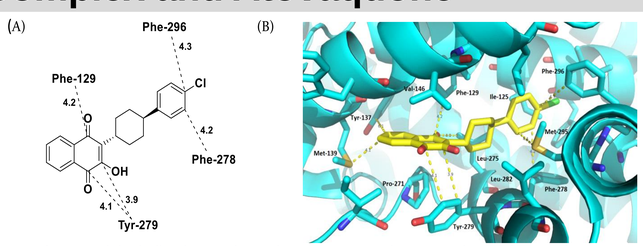

Figure 1. (A) Binding interactions of atovaquone and yeast cyt bc1. (B) Crystal structure of the Qo binding site of yeast cyt bc1 complex (cyan) with atovaquone bound (yellow).

Hypothesis

By analyzing binding interactions between atovaquone and yeast cytochrome bc_1 *in silico*, and highlighting addition protein-drug interactions not known between atovaquone and cytochrome bc_1, potential inhibitors atovaquone-resistant and multi-drug-resistant *P. falciparum* strains will be identified.

Hydroxy-1,4-naphthoquinones

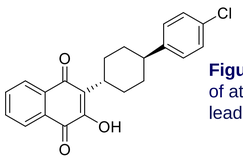

Figure 2. Structure of atovaquone, our lead for design.

Using a crystal structure of a yeast cytochrome bc_1 complex (4PD4), over 30 compounds were explored. Six of the most promising *in silico* leads were synthesized. Three representative poses are shown below in **Figures 4-6**.

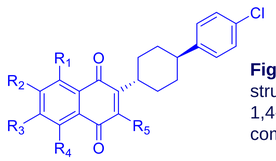

Figure 3. General structure of hydroxy-1,4-naphthoquinone compounds, **SJK 1-6.**

Cytochrome bc_1 and Hydroxy-1,4-naphthoquinones

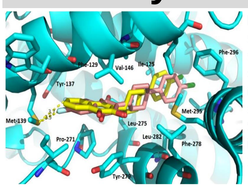

Figure 4. Crystal structure of Qo binding site of yeast cyt bc1 complex (cyan) bound to atovaquone (yellow) and **SJK-3** (pink). **SJK-3** is also interacting with Met 139.

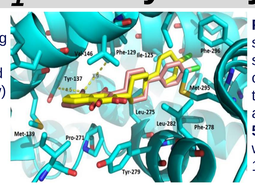

Figure 5. Crystal structure of Qo binding site of yeast cyt bc1 complex (cyan) bound to atovaquone (yellow) and **SJK-5** (pink). **SJK-5** is also interacting with Phe 129 amd Tyr 137.

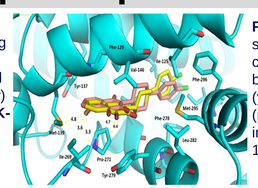

Figure 6. Qo binding site of yeast cyt bc1 complex (cyan) bound to atovaquone (yellow) and **SJK-6** (pink). **SJK-6** is also interacting with Met 139 and Ile 269.

Phenoxy-phenyl-naphthoquinones

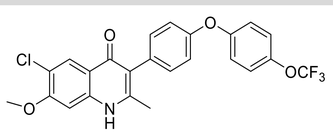

Figure 7. Structure of ELQ-300, a known *P. falciparum* and *P. vivax* inhibitor.

After optimizing the naphthoquinone ring during design of the **SJK** series, the **IHK** series was synthesized by employing structural elements from a known antimalarial along **SJK** series design elements.

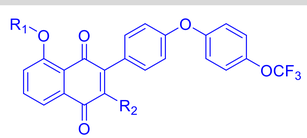

Figure 8. General structure of phenoxy-phenyl-naphthoquinone compounds, **IHK 1-6**.

Anti-plasmodial Results

Compound	*P. falciparum* D6 IC_{50} (μM)	*P. falciparum* W2 IC_{50} (μM)	*P. falciparum* Dd_2 IC_{50} (μM)	*P. falciparum* C_2B IC_{50} (μM)	VERO IC_{50}
Artemisinin	0.035	0.035	N/A	N/A	>0.843
Chloroquine	0.024	0.024	N/A	N/A	>0.744
Atovaquone	**8.2×10^{-4}**	**8.2×10^{-4}**	**2.16×10^{-7}**	**9.442**	>0.130
SJK-1	8.740	8.740	0.734	0.091	>12.31
SJK-2	1.736	1.736	0.372	0.348	>11.82
SJK-3	0.201	0.201	0.024	**0.056**	6.437
SJK-4	0.268	0.268	0.107	3.003	>11.92
SJK-5	**2.6×10^{-3}**	**2.63×10^{-3}**	**1.83×10^{-7}**	0.087	>0.125
SJK-6	**4.5×10^{-3}**	**4.5×10^{-3}**	2.93×10^{-7}	0.356	>0.125
IHK-1	>4.76	>4.76	N/A	N/A	>4.76
IHK-2	4.59	>4.76	N/A	N/A	>4.76
IHK-3	>4.76	3.91	N/A	N/A	>4.76
IHK-4	>4.76	>4.76	N/A	N/A	>4.76
IHK-5	>4.76	>4.76	N/A	N/A	>4.76
IHK-6	>4.76	4.33	N/A	N/A	>4.76

Table 1. *In vitro* anti-plasmodial activity including atovaquone and multi-drug resistant strains.

- **SJK-5** and **6** displayed activity against D6 (chloroquine-resistant) and W2 (chloroquine-senstive) strains.
- **SJK-5** also demonstrated activity against Dd_2 (multi-drug resistant) strain.
- All **SJK** series displayed activity against atovaquone-resistant C_2B isolates.
- **IHK** series compounds did not exhibit significant anti-plasmodial activity.

Antibacterial Results

Compounds	MRSa (μM)	*E. coli* (μM)	*P. aeruginosa* (μM)	*K. pneumoniae* (μM)	VRE (μM)
FLU	>326.51	>326.51	>326.51	>326.51	>326.51
AMB	>108.21	>108.21	>108.21	>108.21	>108.21
CIPRO	>30.18	<0.03	**0.48**	>30.18	>30.18
Vanco	**0.93**	>69.00	>69.00	>69.00	>69.00
METH	27.49	>262.87	135.00	>262.87	>262.87
CEFO	25.07	>219.22	**7.99**	>219.55	>219.55
MERO	7.65	37.57	32.11	196.50	>260.78
Atovaquone	>54.52	>54.52	>54.52	>54.52	>54.52
SJK-1	>51.70	>51.70	>51.70	>51.70	>51.70
SJK-2	>49.65	>49.65	>49.65	>49.65	>49.65
SJK-3	>49.65	>49.65	>49.65	>49.65	>49.65
SJK-4	>50.09	>50.09	>50.09	>50.09	>50.09
SJK-5	>52.51	>52.51	**3.20**	>52.51	>52.51
SJK-6	>52.51	>52.51	**3.47**	>52.51	>52.51
IHK-1	**1.25**	NA	NA	NA	NA
IHK-4	9.00	NA	NA	NA	NA

Table 2. *In vitro* anti-bacterial compound activity including anti-MRSa activity.

- **SJK-5** and **6** displayed activity against *p. aeruginosa in vivo*
  - No other significant efficacy antibacterial activity for hydroxy-1,4-naphthoquinones was observed.
- **IHK-1** and **4** displayed activity against MRSa
  - **IHK-1** performed similarly to vancomycin *in vivo*.
- No compound leads for *E. coli*, *K. penumoniae*, or VRE were discovered.

Conclusion and Future Directions

- Combined rational-*in silico* approach for identified anti-malarial leads
- Antibacterial leads for both *P. aeruginosa* and *methicillin-resistant Staphylococcus aureus* were identified
- Our **future directions**
  - Synthesis of 2nd generation antimalarial library based on **SJK-5** and **6**
  - Synthesis of **IHK-1** and **4** analogues and assessment of activity against *P. aeruginosa* and *methicillin-resistant Staphylococcus aureus*.

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