An Ultra-long-acting Atovaquone Prodrug Formulation for Malaria Chemoprophylaxis

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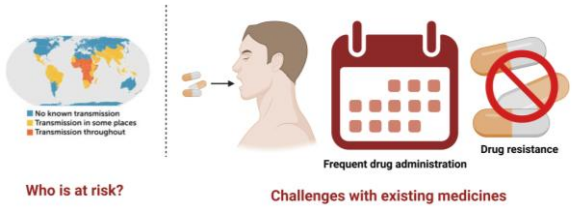
BACKGROUND

Malaria remains a life-threatening disease to public health.



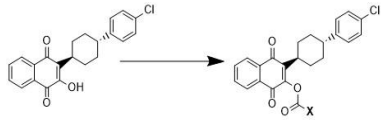
Challenges:
- Frequent drug administration
- Development of drug resistance
- Limited vaccine efficacy

A Long-acting (LA) antimalarial therapy is not available

Drug of Choice: Atovaquone

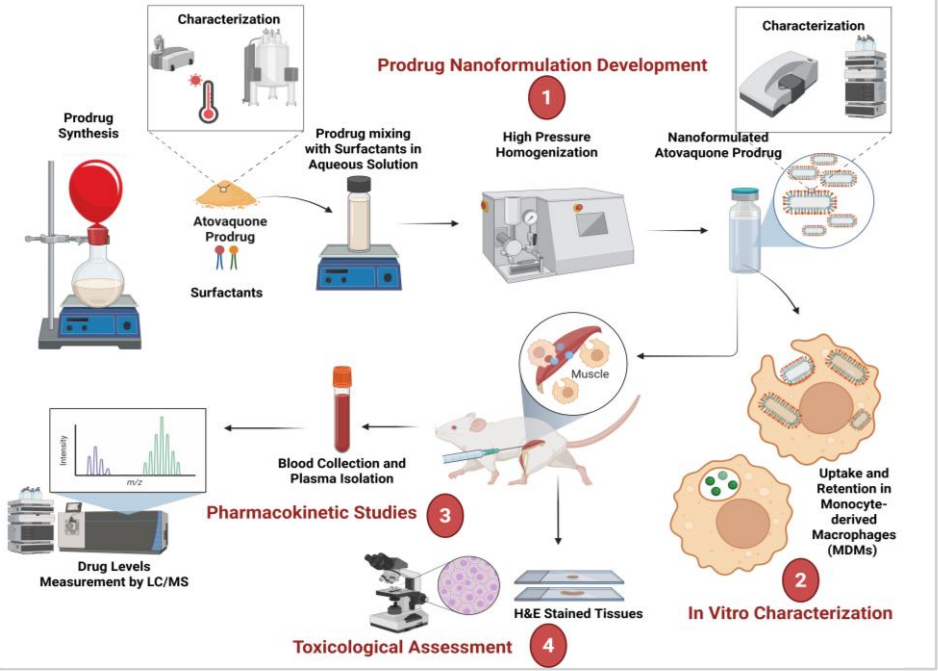
- Atovaquone (ATQ) is an antimalarial drug used for prevention.
- Its inherent molecular properties (hydrophobicity, slow systemic clearance and few drug-drug interactions) make it a promising candidate for LA malaria chemoprophylaxis therapy.

Prodrug Strategy



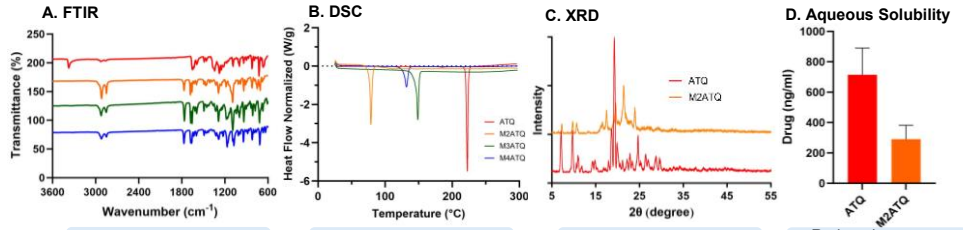
The aim is to develop an ATQ prodrug formulation with extended dosing intervals to maximize malaria prevention.

APPROACH



RESULTS

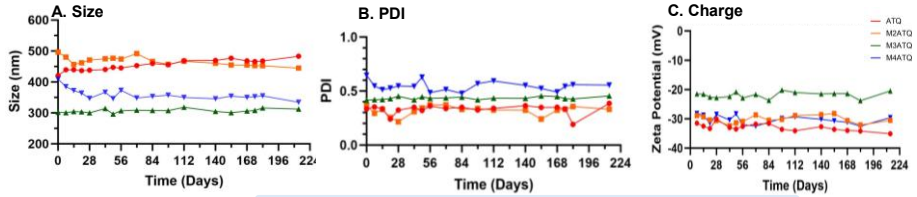
1 Physicochemical Characterization



Structure confirmation | No polymorphs | Crystalline prodrug | Reduced aqueous solubility

Figure 1. **(A)** Fourier-transform infrared spectra (FT-IR) overlay of ATQ, M2ATQ, M3ATQ and M4ATQ. In addition to Nuclear Magnetic Resonance and mass spectrometry, absorption bands in FT-IR spectrum at 2915 cm^{-1} and 2850 cm^{-1} in the prodrug spectra confirmed the chemical modification of ATQ. **(B)** Differential Scanning Calorimetry (DSC) thermograms of prodrugs confirmed melting points and suggested the absence of thermally distinct polymorphs. **(C)** The crystallinity and **(D)** Aqueous solubility of the lead candidate M2ATQ was determined by X-ray diffraction and HPLC, respectively.

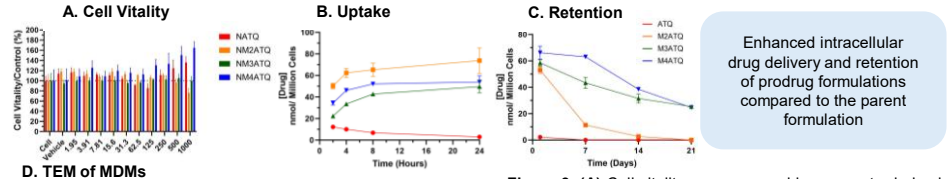
2 Nanoformulation Stability



Nanoformulations were stable for at least six months

Figure 2. The stability of ATQ and prodrug nanoformulations was evaluated over 214 days following manufacture as determined by **(A)** size (nm), **(B)** Polydispersity index (PDI), and **(C)** Zeta potential (mV).

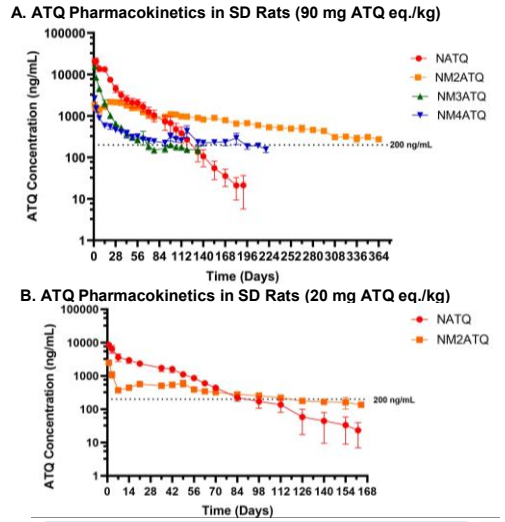
3 In Vitro Studies



Enhanced intracellular drug delivery and retention of prodrug formulations compared to the parent formulation

Figure 3. **(A)** Cell vitality was assessed in monocyte-derived macrophages (MDMs) by MTT assay at 24 h after NATQ, NM2ATQ, NM3ATQ or NM4ATQ treatment. **(B)** Uptake and **(C)** Retention in MDMs. **(D)** Transmission Electron Microscopy (TEM) images of MDMs treated with 50 μM of NATQ, NM2ATQ, NM3ATQ or NM4ATQ.

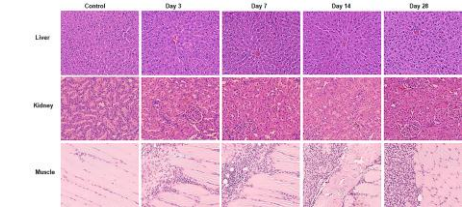
4 Pharmacokinetic Studies



NM2ATQ improved pharmacokinetic profile of ATQ

Figure 4. Sprague Dawley rats were administered a single intramuscular (IM) injection of 90 mg ATQ equivalent/kg of either NATQ, NM2ATQ, NM3ATQ or NM4ATQ **(A)** The lead (NM2ATQ) and parent (NATQ) formulations were further screened at single injections of 20 mg ATQ equivalent/kg **(B)**. The dotted line indicates the effective ATQ plasma concentration of 200 ng/ml. Drug levels were determined by LC-MS/MS. The animal numbers in each group are N = 5. Data are presented as mean ± SEM.

5 Toxicological Assessment



NM2ATQ injection was well tolerated

Figure 5. Histopathological evaluation of tissues in Sprague Dawley rats following an IM injection. Tissues were stained with hematoxylin and eosin. Magnification: 20X.

CONCLUSIONS

- ATQ prodrugs exhibited distinct physicochemical properties from the parent drug.
- The prodrug nanosuspensions facilitated enhanced drug delivery and retention in macrophages in vitro compared to NATQ.
- A single intramuscular injection of the lead NM2ATQ nanosuspension at 90 mg and 20 mg ATQ eq./kg sustained plasma ATQ levels above the effective concentration of 200 ng/mL in rats for a year and four months, respectively.
- NM2ATQ nanosuspension improved the PK profile of ATQ, highlighting its potential for extended dosing intervals of every four months to once a year.

ACKNOWLEDGMENTS

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