Targeting the Mycobacterium ulcerans cytochrome bc_1:aa_3 for the treatment of Buruli ulcer

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INTRODUCTION

Buruli ulcer, caused by Mycobacterium ulcerans, leads to skin lesions, extensive tissue damage and long-term disabilities. Current treatment involves prolonged antibiotic use with rifampin which can lead to severe side effects. However, the M. ulcerans strains that belong to the classical lineage from African and Australian origin lost all their terminal electron acceptors except the cyt-bc1:aa3, revealing the vulnerability of this target to chemical inhibition that can be exploited to develop potent drugs.
The drug Q203 targets the respiratory cytochrome bc1:aa3 (cyt-bc1:aa3). Hence, this study aims to investigate Q203 as a potential therapeutic agent targeting the respiratory cytochrome bc1:aa3 complex of M. ulcerans

MATERIALS/METHODS

The cytochrome bc1:aa3 complex was first identified as the key respiratory target in Mycobacterium ulcerans. In vitro assays measured Q203's minimal inhibitory concentrations (MICs) and bactericidal activity using cultured bacterial strains. In vivo studies involved treating M. ulcerans-infected mouse models with Q203 to monitor bacterial load reduction and lesion healing. Histological analysis and dose-response curves were used to evaluate bacterial survival and tissue recovery. Q203 was compared to samples of ND-11176, BDQ in vitro, as well as Rifampicin & Rifampicin + Streptomycin in vivo.

RESULTS

Identifying the drug target

In Mycobacterium ulcerans, a single stop codon, was responsible for the cydAB operon producing a dysfunctional cyt-bd, and hence issues in Oxidative Phosphorylation. This was illustrated in the study via the difference in effectiveness of Q203 on Classical and Ancestral (without) cydAB (seen in Figure 1b)

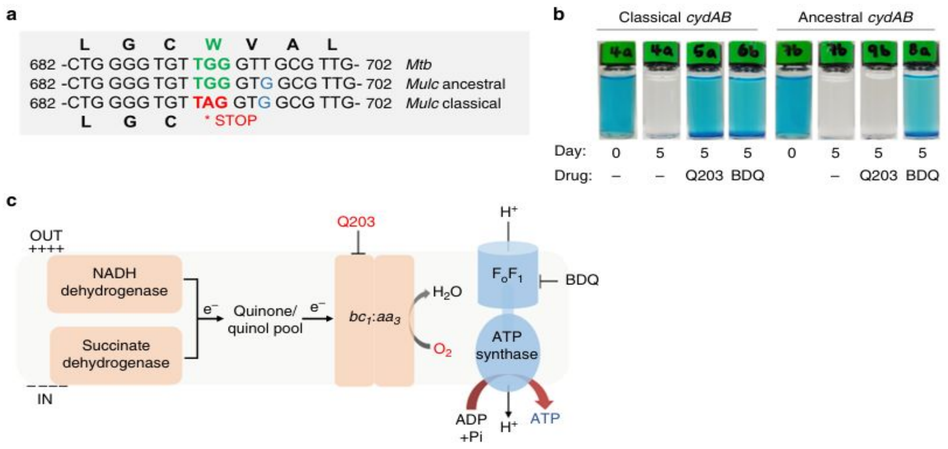

Fig. 1 a,c : *cydAB operon present in classical M. ulcerans strains does not encode a functional cyt-bd, identifying the Oxidative Phosphorylation pathway targets of Q203 and bedaquiline (BDQ)*
Fig 1b: *Q203-treated M. bovis strains complemented with the cydAB operon from classical lineage were incubated with Q203 in sealed tubes containing 0.001% methylene blue used as an oxygen sensor.*

Q203 effectiveness in vitro

In treating the Mycobacterium Ulcerans samples, Q203 outperformed (BDQ) in measured MIC50 values, bacterial growth inhibition and bactericidal potential for both the classical and ancestral strains (*Figure 2a,c,d)*. However, the ancestral strains showed reduced sensitivity to Q203, with MIC50 values 4-8 times higher than the classical strain *(Figure 2b)*.

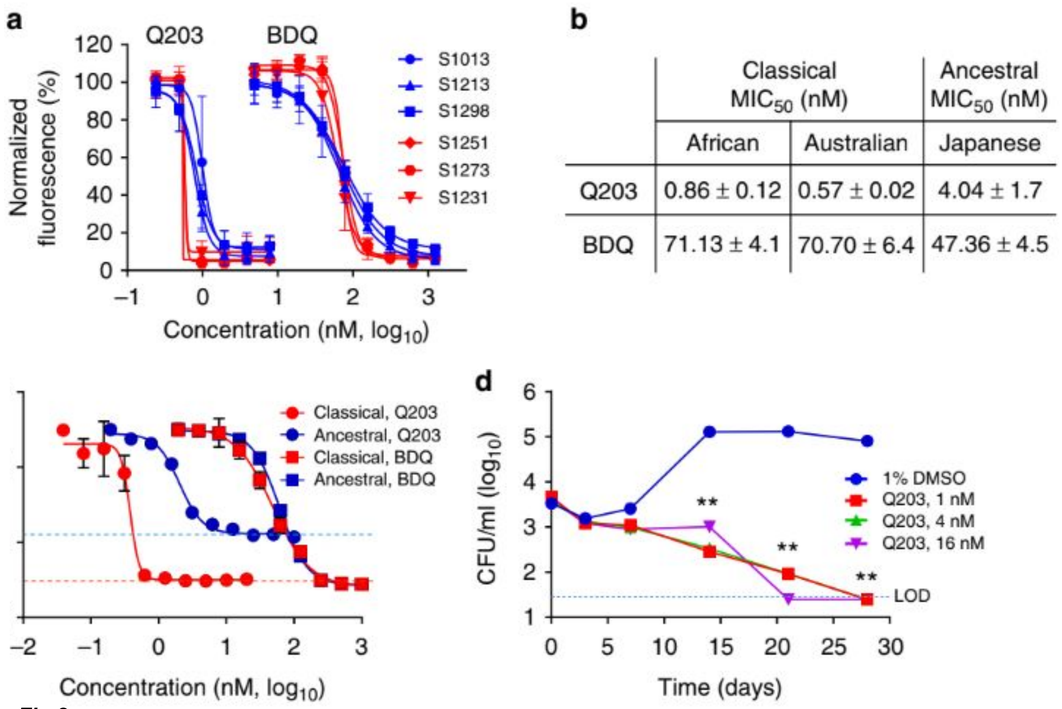

Fig 2
(a) *Growth inhibitory activity of Q203 and BDQ against classical M. ulceran from Africa (blue symbols) and Australia (red symbols).Growth inhibitory activity in Classical vs Ancestral strains measured in MIC50 (b) and OD600 (c). Bactericdal potential was demonstrated via CFU (d)*

Oxygen consumption of Q203 and its analogue

In Mycobacterium Ulcerans populations, Oxygen consumption and ATP levels were also shown to be reduced significantly after administration of Q203, as well as its analogue ND-11176. Supporting the hypothesis that the bc1:aa3 is responsible for its effectiveness. Both Q203 and ND-11176 outperformed BDQ.

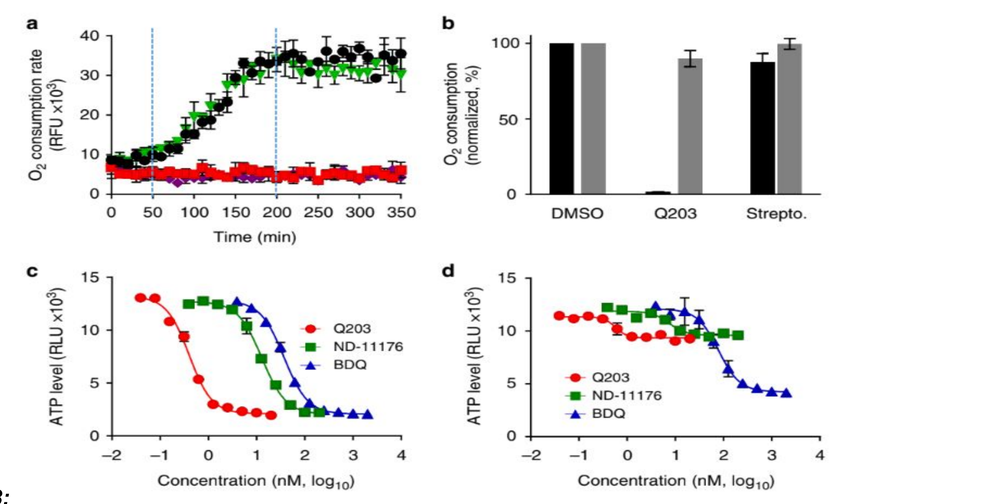

Figure 3:
(a,b) *Oxygen consumption rates in the classical lineage M. ulcerans strain S1013 treated with Q203 (5nM, red squares), ND-11176 (50nM, purple diamonds), streptomycin (5000nM, green triangles) or DMSO (1%, black circles)* ***(b)*** *Effect of Q203 (5nM) or streptomycin (5000nM) treatment on the oxygen consumption rate in the classical strain S1013 (dark grey bars) and the ancestral strain S1325* ***(c,d)*** *Quantification of intracellular ATP levels in the classical strain S1013 (c) or in the ancestral strain S1325 (d)*

Q203 effectiveness in vivo (mice)

The effectiveness of Q203 was also demonstrated in mice models, where it was shown to be more effective than the current clinically used regime (Streptomycin + Rifampicin) as well as Rifampicin alone. This was demonstrated via a greater reduction in CFU and footpad thickness in mice infected with Mycobacterium ulcerans.

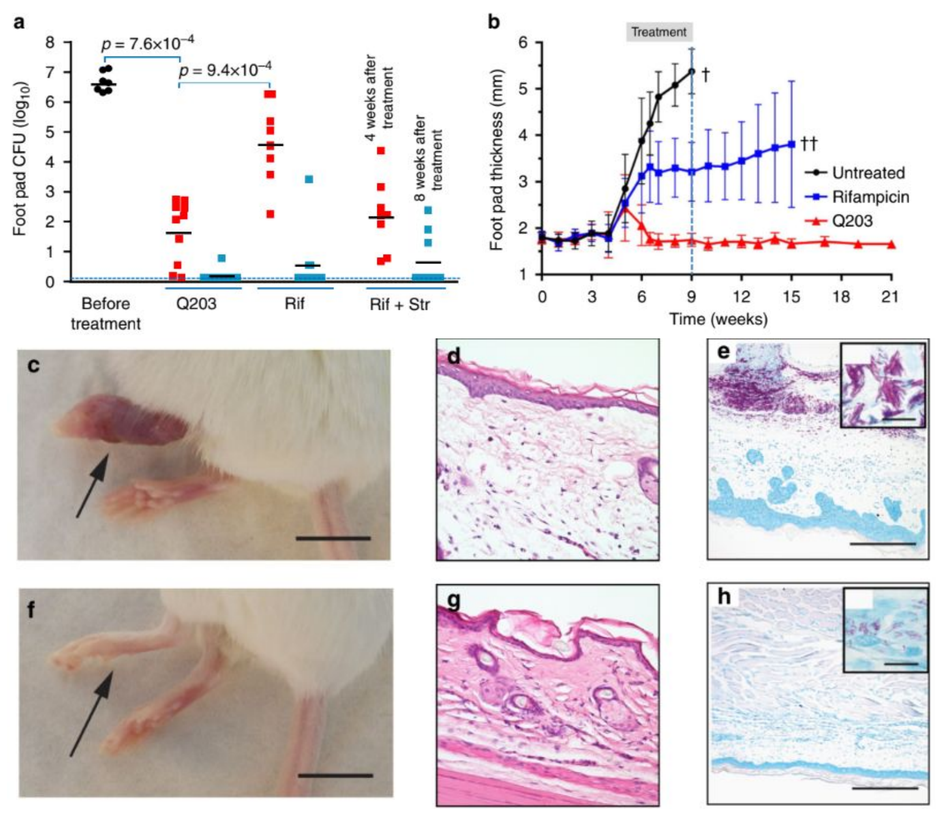

Fig. 4a,b: *Graphical representation of the efficacy of Q203 (via CFU) in the mouse footpad infection model of Buruli ulcer. Q203, rifampicin (Rif) and streptomycin (Str).* ***Fib 4 c, f:*** *Appearance of infected foot at week 9 treated with the vehicle control (c) or with Q203 (f). Black arrows shows the site of infection.* ***Fig 4 d,g:*** *Microscopic differences between untreated (d,e) and treated (g,h). (samples e and h were AFB stained)*

CONCLUSION

This study highlights the potential of targeting the respiratory cyt-bc1:aa3 complex as a novel therapeutic approach for Buruli ulcer (BU). Classical *Mycobacterium ulcerans* strains, exhibit exquisite sensitivity to Q203 due to the loss of the cyt-bd terminal oxidase, rendering their respiration solely dependent on the cyt-bc1:aa3 complex. This study demonstrates the effectiveness of Q203 via in vitro and in vivo populations, as well as isolated the respiratory targets responsible for said effectiveness. These findings emphasize the importance of leveraging natural metabolic adaptations in pathogens to develop more effective, safer, and patient-friendly treatment options, with Q203 offering significant promise for future clinical trials.

REFERENCES

1. Scherr, N., Bieri, R., Thomas, S. S., Chauffour, A., Kalia, N. P., Schneide, P., Ruf, M. T., et al. (2018). Targeting the *Mycobacterium ulcerans* cytochrome bc1:aa3 for the treatment of Buruli ulcer. *Nature Communications*, 9, 5370. DOI: 10.1038/s41467-018-07804-8.

2. Yeboah-Manu, D., et al. (2018). *Mycobacterium ulcerans* disease: Current understanding and future directions. *PLoS Neglected Tropical Diseases*, 12(3), e0006363. DOI: 10.1371/journal.pntd.0006363.

3. World Health Organization (2023). Buruli ulcer (Mycobacterium ulcerans infection): Fact Sheet. Retrieved from https://www.who.int.

4. Converse, P. J., Nuermberger, E. L., Almeida, D. V., Grosset, J. H. (2011). Treating *Mycobacterium ulcerans* disease: From preclinical studies to implementation research. *PLoS Neglected Tropical Diseases*, 5(10), e1380. DOI: 10.1371/journal.pntd.0001380.