Analysis of the binding of imidazopyridine amides to the *Mycobacterium smegmatis* $CIII_2CIV_2$ respiratory supercomplex

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Introduction

Mycobacterial lung infections are caused by various mycobacteria. One example is *Mycobacterium tuberculosis* (*Mtb)* which causes tuberculosis (TB), one of the oldest respiratory diseases and a leading cause of death worldwide.[1, 2]. Treatment of mycobacterial infections is challenging due to the continuous emergence of resistant strains and the difficulty of killing non-replicating (dormant) bacilli. Cellular respiration is the main source of ATP production in all organisms including mycobacteria. The dependence of both replicating and non-replicating mycobacteria on respiration for energy production makes it a promising drug target with the necessity of organismal selectivity. Telacebec, also known as Q203[3], is an imidazopyridine amide (IPA) that targets $CIII_2CIV_2$ of mycobacteria, which replaces the canonical CIII and CIV.[4] To gain a better understanding of the molecular mechanism of action, oxygen consumption assays were designed and performed to test the activity and specificity of a series of synthesized IPA analogues and Q203 against purified *M. smegmatis* $CIII_2CIV_2$. *M. smegmatis (Msmeg)*, a fast-growing non-pathogenic mycobacterium, is used as a model due to the structural similarities with *Mtb*. The activity of Q203 and selected IPAs was tested against *Bos taurus* mitochondrial CIII to confirm their specificity to mycobacteria.

1. *M. smegmatis* $CIII_2CIV_2$ activity

Assay principle. The inhibition of $CIII_2CIV_2$ was tested using a clark-type oxygen electrode. 2,3-dimethyl[1,4]naphthoquinone (DMW) was used as the electron donor and NADH dehydrogenase enzyme from *Caldalkalibacillus thermarum* (NDH-2) was added to the reaction mixture to reduce DMW to $DMWH_2$. The reaction was initiated by the addition of NADH to the oxygen electrode chamber.

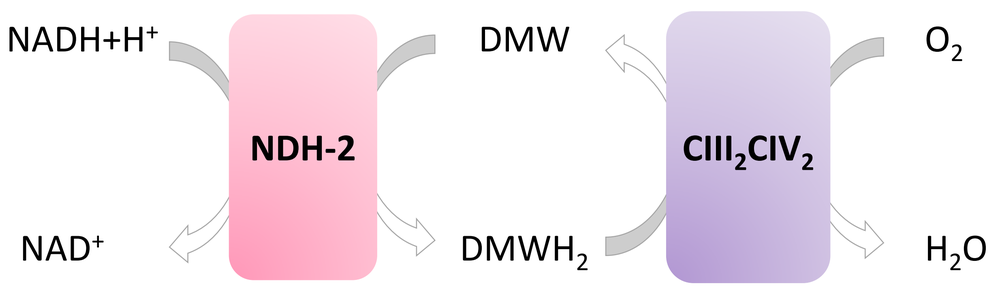

Figure 1. A diagram illustrating the oxygen consumption assay principle.

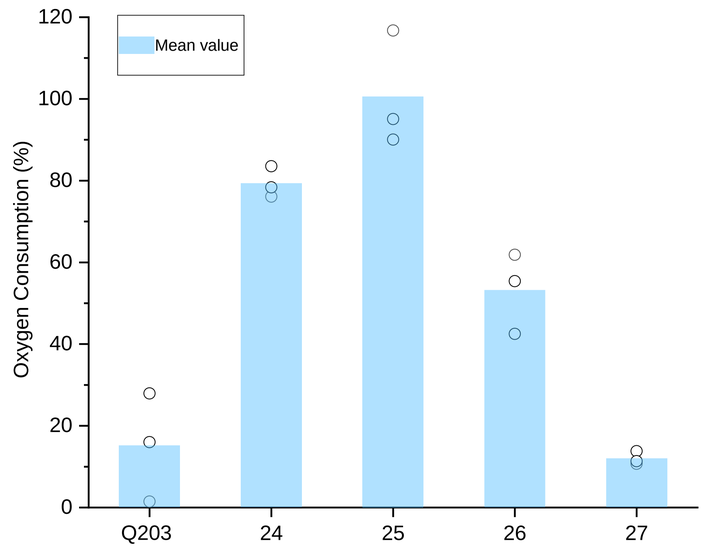

Figure 2. Average % activity against *M. smegmatis* $CIII_2CIV_2$. The graph shows the average % activity of Q203 and compounds **24-27**. At the tested concentration (10 µM), compound **27** showed 88 ± 7% inhibition of $CIII_2CIV_2$ activity, which is similar to inhibition by Q203 (85 ± 13 %). compound **26** showed 50 % inhibition, while compound **24**[5] showed only 20 % inhibition and compound **25**[5] showed very weak to no binding. (mean +/- s.d., n = 3 independent assays)

2. Mitochondrial CIII activity

The activity of some of the IPAs against *Bos taurus* mitochondrial cytochrome bc1 complex (CIII) was tested to verify their specificity against mycobacterial $CIII_2CIV_2$. The IPAs chosen were Q203, the lead compound, **27** which showed strong binding in the above mentioned *M. smegmatis* $CIII_2CIV_2$ activity assays, and **24**[4] which despite its reported high potency in literature was inactive in our binding assay mentioned above. Oxygen consumption assays, where Q203, **24**[4], or **27** were incubated with sub-mitochondrial particles (SMPs, **Figure 3**) at concentrations of 10 µM and 1 µM, were performed. Rotenone (complex I inhibitor), antimycin A (complex III inhibitor) and KCN (complex IV inhibitor) were used as positive controls. The SMPs were formed by the sonication of purified bovine mitochondrial vesicles.

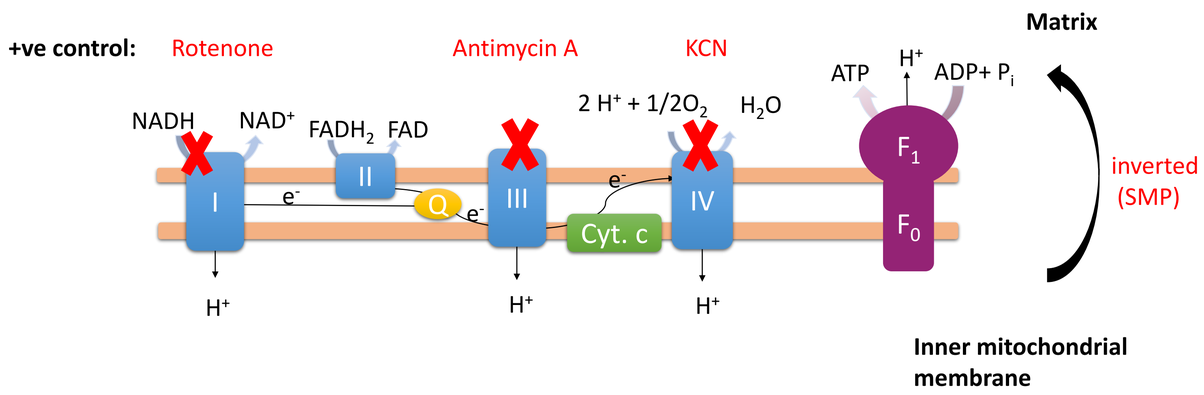

Figure 3. A diagram illustrating the submitochondirial membrane (SMPs) and the inhibitors used as positive controls..

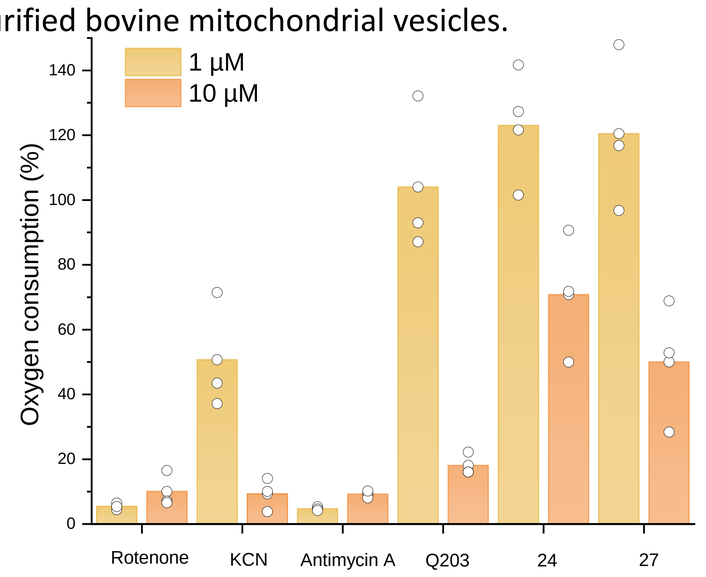

Figure 4. Activity of Q203, 24 and 27 at [10 µM] and [1 µM] in sub-mitochondrial particles. The graph shows that Q203, **24**[4], and **27** inhibit complex III at 10 µM; 80%, 30% 50% inhibition respectively, while at 1 µM they show no inhibition. (mean +/- s.d., n = 3 independent assays)

Conclusion

The assay results generally supported the structure activity relationship information obtained from the structure of *Msmeg* $CIII_2CIV_2$ bound to Q203[6] and *Mtb* $CIII_2$ model[7]. For example, a halogen substituent at C6/C7 of the IP moiety is important for $CIII_2CIV_2$ inhibitory activity and *in silico* models showed that a halogen bond[8] can be formed with the carbonyl of Leu166 in *Msmeg*[6] and with Tyr164 in *Mtb*[7]. None of the compounds tested showed significant activity against *bovine* mitochondrial complex III, suggesting safe use in humans.

Acknowledgement

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