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# Selectivity of TMC207 towards Mycobacterial ATP Synthase Compared with That towards the Eukaryotic Homologue[▽]

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**The diarylquinoline TMC207 kills *Mycobacterium tuberculosis* by specifically inhibiting ATP synthase. We show here that human mitochondrial ATP synthase (50% inhibitory concentration [$IC_{50}$] of >200 μM) displayed more than 20,000-fold lower sensitivity for TMC207 compared to that of mycobacterial ATP synthase ($IC_{50}$ of 10 nM). Also, oxygen consumption in mouse liver and bovine heart mitochondria showed very low sensitivity for TMC207. These results suggest that TMC207 may not elicit ATP synthesis-related toxicity in mammalian cells. ATP synthase, although highly conserved between prokaryotes and eukaryotes, may still qualify as an attractive antibiotic target.**

A new series of compounds, the diarylquinolines, was reported to be highly active against *Mycobacterium tuberculosis* (3). TMC207, the lead compound of the diarylquinoline series, displays MICs of 30 nM for *M. tuberculosis* and 15 nM for *Mycobacterium smegmatis*. We recently demonstrated that TMC207 targets ATP synthase, the enzyme responsible for ATP production by oxidative phosphorylation (11). The inhibition of ATP synthase by TMC207 was highly specific, with a 50% inhibitory concentration ($IC_{50}$) for this enzyme corresponding to the MIC for bacterial growth inhibition (11). This compound, which efficiently kills replicating as well as dormant mycobacteria (12, 18), is currently in clinical development in phase IIb trials in patients with multidrug-resistant tuberculosis.

An important factor to consider for a new antibacterial drug is the lack of a eukaryotic homologue of the target, as inhibition of a homologous enzyme could lead to toxicity and safety concerns in humans. In the case of TMC207, the target enzyme ATP synthase is essential for survival in higher organisms, as it supplies cells with the bulk of their ATP via oxidative phosphorylation (20). ATP synthase is evolutionarily strongly conserved among prokaryotes and eukaryotes. Universally, ATP synthesis is coupled to the flow of protons from the intercristae region in mitochondria and the periplasmic space in bacteria to the mitochondrial matrix and the bacterial cytoplasm, respectively. Subunit c of ATP synthase, forming a membrane-spanning oligomer, is essential for this proton transport (8).

TMC207 binds to subunit c of mycobacterial ATP synthase (11). Several natural compounds, such as oligomycin and venturicidin, are known to block ATP synthase action by interaction with subunit c. However, these compounds are not selective and inhibit ATP synthase not only in bacteria but also in mitochondria (14, 15). This lack of selectivity prevents their clinical usage due to toxicity issues and fatality concerns. Mitochondrial toxicity is a major concern in the clinical development of new drugs, as it may lead to disease conditions, such as pancreatitis, peripheral neuropathy, and cardial or skeletal myopathies (1, 21).

Hence, it is of key importance to investigate the selectivity of TMC207 towards mycobacterial ATP synthase compared with that towards mitochondrial ATP synthase.

*Mycobacterium smegmatis* $mc^2155$ was cultured and inverted membrane vesicles were prepared as described previously (2, 11). The human ovarian cancer cell line OVCAR3 was grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 100 units/ml penicillin-streptomycin. Mitochondria and submitochondrial particles (SMPs) were isolated and prepared according to methods outlined previously (6, 9, 22). ATP synthesis activity by human mitochondria and mycobacterial membrane vesicles was measured as described previously (6, 9, 11). Mouse liver mitochondria and mitoplasts were isolated and prepared from M18 mice as described previously (4, 15). The method of Smith (19) was used to isolate bovine heart mitochondria. Mitochondria or mitoplast oxygen consumption rates were monitored according to the methods from references 5 and 16.

First, we monitored the effect of TMC207 on ATP synthesis in isolated mitochondria from a human cancer cell line. No effect of TMC207 on ATP production was observed at nanomolar concentrations; extremely high concentrations of 200 μM compound lead to approximately 35% inhibition (Fig. 1A). *N*,*N*′-dicyclohexyl-carbodiimide (DCCD; 5 μM) and oligomycin (1 μM), two nonselective ATP synthase inhibitors, both suppressed ATP synthesis by >90% (Fig. 1A). In contrast, ATP synthase within inverted membrane vesicles of *M. smegmatis* was efficiently inhibited by nanomolar concentrations of TMC207. Half-maximal inhibition was achieved with 10 nM

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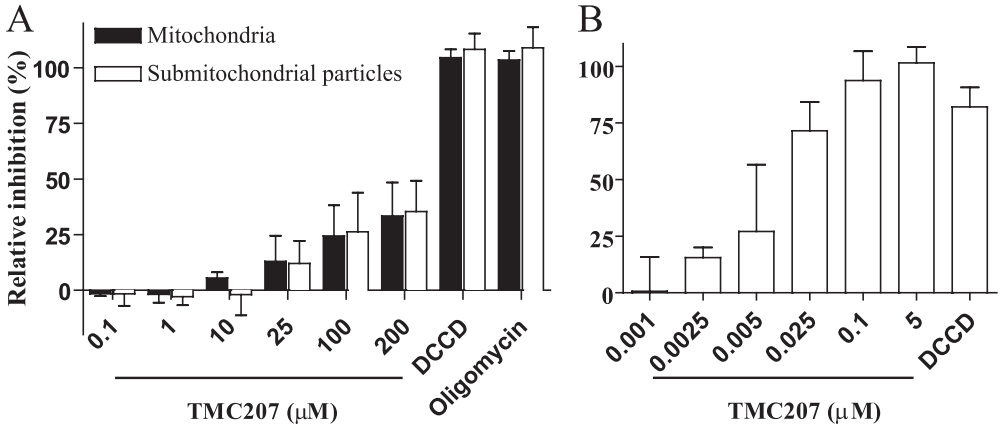


FIG. 1. Effect of TMC207 on ATP synthesis by mitochondria from a human cell line. ATP synthesis in the presence of TMC207 was measured for mitochondria (250 μg/ml) and SMPs (150 μg/ml) from a human cancer cell line (A) and compared to that for inverted membrane vesicles of *Mycobacterium smegmatis* (B). Samples were incubated at 25°C for 1 h in the presence of an ADP-regenerating system, and produced ATP was quantified spectrophotometrically by monitoring the oxidation of glucose-6-phosphate with $NADP^+$. As controls, DCCD (100 μM for *M. smegmatis* and 5 μM for human mitochondria and SMPs) and oligomycin (1 μM) were used.

TMC207; virtually complete inhibition was achieved in the presence of 100 nM compound (Fig. 1B). From the $IC_{50}$s obtained for human ($IC_{50}$, >200 μM) and mycobacterial ($IC_{50}$, 0.01 μM) ATP synthase, a high selectivity index of >20,000 for TMC207 was calculated. TMC207 has a strongly hydrophobic core structure (3) and may get trapped in the mitochondrial outer membrane. For this reason, we also investigated the effect of TMC207 on SMPs, mitochondria from which the outer membrane was removed by sonication treatment. However, as observed for whole mitochondria, human SMPs showed only very low sensitivity for TMC207, with an $IC_{50}$ of >200 μM (Fig. 1A). Thus, the lack of susceptibility of human ATP synthase cannot be accounted for by a permeability barrier function of the mitochondrial outer membrane.

We then determined the effect of TMC207 on oxygen consumption by mitochondria freshly isolated from mouse liver and bovine heart tissue. In isolated mitochondria, any inhibition of ATP synthase or the respiratory chain enzyme complexes will cause decreased oxygen consumption. Here, for both mouse liver and bovine heart mitochondria, no significant effect on oxygen consumption was measured for TMC207, even in the presence of high (175 μM) concentrations (Fig. 2). As a control, oligomycin efficiently inhibited oxygen consumption by >80% in mouse liver and >50% in bovine heart mitochondria. Furthermore, ATP synthase and respiratory function in mitoplasts, mouse liver mitochondria from which the outer membrane was removed by mild osmotic shock, were not significantly inhibited by TMC207 (Fig. 2).

Taken together, mitochondria from human cells, mouse liver, and bovine heart show only very low sensitivity for TMC207. The high selectivity index for TMC207 indicates that the compound is very specific and unlikely to induce target-based toxicity in mammalian cells. In drug development, compounds with a selectivity index of >1,000 are regarded as promising candidates for clinical development. TMC207 may thus be the first highly selective ATP synthase inhibitor with the potential to treat a bacterial infection. In a phase I clinical study with healthy volunteers, short-term administration of TMC207 in humans was found to be safe and well tolerated without serious adverse effects (3). Our results provide the basis for a rational explanation of the preliminary clinical safety observed with this compound.

It has previously been shown that the point mutations A63P and I66M in subunit c (Fig. 3) lead to acquired resistance in *M. tuberculosis* (3, 17). Furthermore, in certain mycobacterial species like *M. xenopi*, *M. shimoidei*, and *M. novocastrense*, the naturally occurring genetic polymorphism of A63M results in intrinsic resistance (10). Docking studies based on binding energy minimization have suggested that E61, A63, and I66

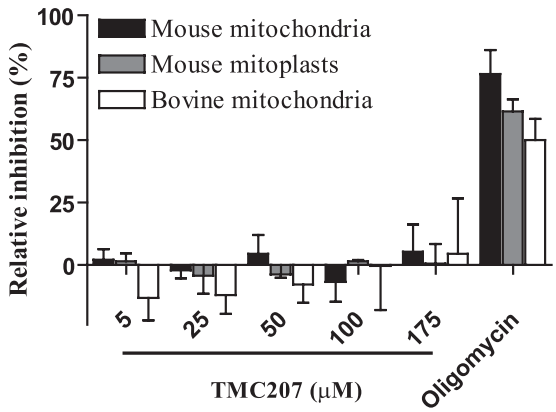


FIG. 2. Effect of TMC207 on respiratory function in mitochondria isolated from mouse and bovine tissue. Oxygen consumption coupled to ATP synthesis in the presence of TMC207 was measured for mitochondria from mouse liver, mitoplasts from mouse liver, and mitochondria from bovine heart (each at a final protein concentration of 1 mg/ml). The oxygen concentration was measured using a Clark electrode at 37°C in a medium with 20 mM Tris-HCl, pH 7.3, 85 mM KCl, 5 mM $KH_2PO_4$, 2.3 mM $MgCl_2$, 25 mM creatine, and 25 mM phosphocreatine in the presence of an ADP-regenerating system and the indicated concentrations of TMC207. The membrane was energized by an addition of succinate, and complex I was inhibited by rotenone. Inhibition of ATP synthase and respiratory chain enzymes was determined as a decrease in the state III oxygen consumption rate. As a control, oligomycin (0.6 μM) was used. Each graph shows mean values of three independent experiments with standard deviations.

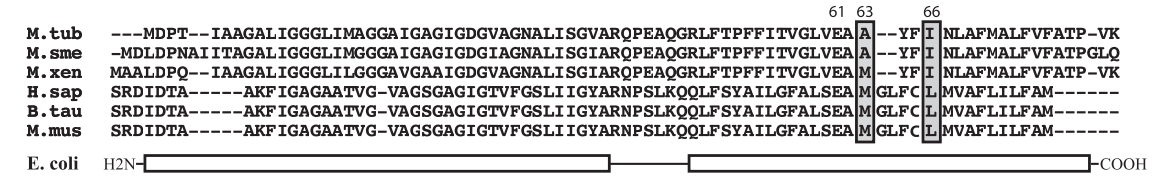


FIG. 3. Multiple sequence alignment for subunit c of ATP synthase. Amino acid sequences of ATP synthase subunit c from mycobacteria and eukaryotic species are compared. Species abbreviations: M.tub, *M. tuberculosis* (Swiss-Prot accession no. Q10598); M.sme, *M. smegmatis* (EMBL accession no. AJ862722); M.xen, *M. xenopi* (GenBank accession no. DQ306893); H.sap, *Homo sapiens* (residue 60 to 136; RefSeq accession no. NM_001002027); B.tau, *Bos taurus* (bovine, residue 60 to 136; RefSeq accession no. NP_788822); and M.mus, *Mus musculus* (mouse, residue 60 to 136; RefSeq accession no. NM_007506). For comparison, the two transmembrane helices found in the structure of the *Escherichia coli* enzyme are indicated below. The N-terminal part of the mitochondrial precursor sequence for the bovine and mouse subunit c is not shown. Amino acid residues found to be important for drug sensitivity in *M. tuberculosis* (positions 63 and 66, numbering for *M. tuberculosis*) are indicated by boxes. The glutamic acid residue E61 is an essential residue for proton translocation.

contribute to a binding pocket for TMC207 in the membrane-spanning region of subunit c (7). Amino acid changes or natural polymorphisms in this binding pocket may cause steric hindrance and thus prevent efficient binding of the drug. Interestingly, ATP synthases from human, mouse, and bovine mitochondria also display a methionine at position 63 of subunit c, and this polymorphism on its own may account for the lack of inhibition of ATP synthase in mitochondria from these organisms. Although in humans three different isoforms of subunit c are known (13), no tissue-specific sensitivity for TMC207 can be expected, as these isoforms represent the same mature protein and differ only in the mitochondrial import sequence.

Targeting the bacterial energy metabolism, despite the fact that the enzymes involved are conserved among prokaryotes and eukaryotes, is a valid approach and may help to combat the increased emergence of drug resistance. More generally, our results illustrate that in drug discovery, a protein should not be disregarded a priori as a potential drug target because of the existence of a human homologue.

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## REFERENCES

1. **Amacher, D. E.** 2005. Drug-associated mitochondrial toxicity and its detection. Curr. Med. Chem. **12:**1829–1839.
2. **Andrew, P. W., and I. S. Roberts.** 1993. Construction of a bioluminescent mycobacterium and its use for assay of antimycobacterial agents. J. Clin. Microbiol. **31:**2251–2254.
3. **Andries, K., P. Verhasselt, J. Guillemont, H. W. H. Göhlmann, J. M. Neefs, H. Winkler, J. Van Gestel, P. Timmerman, M. Zhu, E. Lee, P. Williams, D. de Chaffoy, E. Huitric, S. Hoffner, E. Cambau, C. Truffot-Pernot, N. Lounis, and V. Jarlier.** 2005. A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. Science **307:**223–227.
4. **Bunney, T. D., H. S. van Walraven, and A. H. de Boer.** 2001. 14-3-3 protein is a regulator of the mitochondrial and chloroplast ATP synthase. Proc. Natl. Acad. Sci. USA **98:**4249–4254.
5. **Ciapaite, J., G. Van Eikenhorst, S. J. L. Bakker, M. Diamant, R. J. Heine, M. J. Wagner, H. V. Westerhoff, and K. Krab.** 2005. Modular kinetic analysis of the adenine nucleotide translocator-mediated effects of palmitoyl-CoA on the oxidative phosphorylation in isolated rat liver mitochondria. Diabetes **54:**944–951.
6. **Cortés-Hernández, P., M. E. Vázquez-Memije, and J. J. García.** 2007. ATP6 homoplasmic mutations inhibit and destabilize the human $F_1F_0$-ATP synthase without preventing enzyme assembly and oligomerization. J. Biol. Chem. **282:**1051–1058.
7. **de Jonge, M. R., L. H. Koymans, J. E. Guillemont, A. Koul, and K. Andries.** 2007. A computational model of the inhibition of Mycobacterium tuberculosis ATPase by a new drug candidate R207910. Proteins **67:**971–980.
8. **Fillingame, R. H., and O. Y. Dmitriev.** 2002. Structural model of the transmembrane $F_0$ rotary sector of $H^+$-transporting ATP synthase derived by solution NMR and intersubunit cross-linking in situ. Biochim. Biophys. Acta **1565:**232–245.
9. **García, J. J., I. Ogilvie, B. H. Robinson, and R. A. Capaldi.** 2000. Structure, functioning and assembly of the ATP synthase in cells from patients with the T8993G mitochondrial DNA mutation. Comparison with the enzyme in $Rho^0$ cells completely lacking mtDNA. J. Biol. Chem. **275:**11075–11081.
10. **Huitric, E., P. Verhasselt, K. Andries, and S. E. Hoffner.** 2007. In vitro antimycobacterial spectrum of a diarylquinoline ATP synthase inhibitor. Antimicrob. Agents Chemother. **51:**4202–4204.
11. **Koul, A., N. Dendouga, K. Vergauwen, B. Molenberghs, L. Vranckx, R. Willebrords, Z. Ristic, H. Lill, I. Dorange, J. Guillemont, D. Bald, and K. Andries.** 2007. Diarylquinolines target subunit c of mycobacterial ATP synthase. Nat. Chem. Biol. **3:**323–324.
12. **Koul, A., L. Vranckx, N. Dendouga, W. Balemans, I. Van den Wyngaert, K. Vergauwen, H. W. Göhlmann, R. Willebrords, A. Poncelet, J. Guillemont, D. Bald, and K. Andries.** 2008. Diarylquinolines are bactericidal for dormant mycobacteria as a result of disturbed ATP homeostasis. J. Biol. Chem. **283:**25273–25280.
13. **Kramarova, T. V., I. G. Shabalina, U. Andersson, R. Westerberg, I. Carlberg, J. Houstek, J. Nedergaard, and B. Cannon.** 2008. Mitochondrial ATP synthase levels in brown adipose tissue are governed by the c-$F_0$ subunit P1 isoform. FASEB J. **22:**55–63.
14. **Matsuno-Yagi, A., and Y. Hatefi.** 1993. Studies on the mechanism of oxidative phosphorylation. Different effects of $F_0$ inhibitors on unisite and multisite ATP hydrolysis by bovine submitochondrial particles. J. Biol. Chem. **268:**1539–1545.
15. **Matsuno-Yagi, A., and Y. Hatefi.** 1993. Studies on the mechanism of oxidative phosphorylation. ATP synthesis by submitochondrial particles inhibited at $F_0$ by venturicidin and organotin compounds. J. Biol. Chem. **268:**6168–6173.
16. **Mildaziene, V., Z. Nauciene, R. Baniene, and J. Grigiene.** 2002. Multiple effects of 2,2′,5, 5′-tetrachlorobiphenyl on oxidative phosphorylation in rat liver mitochondria. Toxicol. Sci. **65:**220–227.
17. **Petrella, S., E. Cambau, A. Chauffour, K. Andries, V. Jarlier, and W. Sougakoff.** 2006. Genetic basis for natural and acquired resistance to the diarylquinoline R207910 in mycobacteria. Antimicrob. Agents Chemother. **50:**2853–2856.
18. **Rao, S. P., S. Alonso, L. Rand, T. Dick, and K. Pethe.** 2008. The protonmotive force is required for maintaining ATP homeostasis and viability of hypoxic, nonreplicating *Mycobacterium tuberculosis*. Proc. Natl. Acad. Sci. USA **105:**11945–11950.
19. **Smith, A. L.** 1967. Preparation, properties, and conditions for assay of mitochondria: slaughterhouse material, small-scale. Methods Enzymol. **10:**81–86.
20. **von Ballmoos, C., G. M. Cook, and P. Dimroth.** 2008. Unique rotary ATP synthase and its biological diversity. Annu. Rev. Biophys. **37:**43–64.
21. **Wallace, K. B., and A. A. Starkov.** 2000. Mitochondrial targets of drug toxicity. Annu. Rev. Pharmacol. Toxicol. **40:**353–388.
22. **Wang, X., E. Perez, R. Liu, L.-J. Yan, R. T. Mallet, and S.-H. Yang.** 2007. Pyruvate protects mitochondria from oxidative stress in human neuroblastoma SK-N-SH cells. Brain Res. **1132:**1–9.