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# New Griselimycins for Treatment of Tuberculosis

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**Griselimycin (GM), a natural product isolated a half century ago, is having a bit of a renaissance. After being known for more than 50 years, it is now being pursued as a treatment for tuberculosis. With the new mechanism of action, excellent in vitro and in vivo activity against sensitive and drug-resistant *Mycobacterium tuberculosis*, and the improved pharmacokinetic properties, the cyclohexyl derivative of GM demonstrates a high translational potential.**

Tuberculosis (Tb), caused by *Mycobacterium tuberculosis* (Mtb), is one of the major global infectious diseases. Even though 9 million cases were reported in 2013, with 1.5 million deaths per year (World Health Organization, 2014), the treatment of the infection is managed by old drugs discovered in the 1950s and 1960s. The regimen starts with a four drug combination consisting of isoniazid, rifampicin, ethambutol, and pyrazinamide for 2 months, followed by the combination of isoniazid and rifampicin for another 4 months. In 2012, after some 40 years of almost no progress in anti-Tb drug discovery, two new drugs were approved: bedaquiline, which targets the ATP synthase, and delamanid, a nitroimidazole that releases toxic nitric oxide when metabolized. Both bedaquiline and delamanid are active against multidrug-resistant (MDR) tuberculosis and thus are able to tackle the huge resistance problem. Some additional compounds are in the pipeline in different stages of clinical trials.

Along with the urgent need to solve the problems with MDR and extremely drug-resistant (XDR) strains, there is also a real necessity to find new antibiotics, which will allow a far shorter treatment with a lower number of drugs. Moreover, the new anti-infectives must work well with other drugs because Tb is often associated with HIV, especially in sub-Saharan Africa (where there were approximately 350,000 death in 2013), and diabetes. Finally, because Tb occurs mostly in low-income countries, the new drugs should be cheap. The bottom line is that new, effective, safe, and cheap anti-Tb drugs with different mechanism of action must be developed to address the significant challenge that Tb poses for public health.

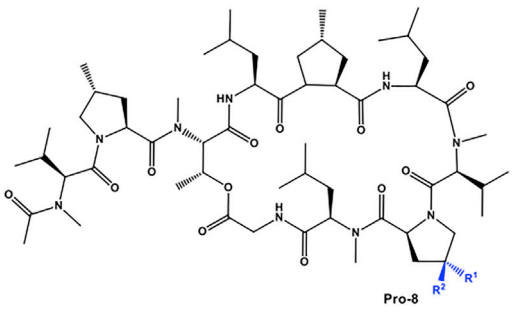


**Figure 1. Structural Formula of Griselimycin**
Alkylation of the proline ring in position 8 reveals an improvement of the pharmacokinetics.

Most molecules that are currently being explored as anti-Tb treatments options fall into the small molecule category, although there is an increasing interest in exploring natural products in this context. Cyclic peptide griselimycin (GM, Figure 1) was isolated in the 1960s from *Streptomyces* and its antibacterial and antimycobacterial activity was evaluated in the early 1970s (Terlain and Thomas, 1971a, 1971b). The early work established that GM has unfavorable pharmacokinetic properties, and, given that rifampicin was approved at that time, the development of GM as an anti-Tb drug was terminated. Recently, Rolf Müller and colleagues decided to revisit GM, given its high activity (Kling et al., 2015). First, they increased the metabolic stability of GM by alkylation of the proline residue in position 8. A cyclohexyl GM (CGM) derivative, obtained via a newly developed total synthesis, was metabolically stable, and the increased lipophilicity enhanced the penetration of the thick

mycobacterial cell wall, resulting in a minimal inhibition concentration of 0.06 μg/ml. The activity against resistant Mtb was similar, indicating the lack of cross-resistances with anti-Tb drugs in use. This also pointed to a different mechanism of action of the GM derivatives from the other mycobactericidal drugs.

The investigation of the self-resistance to GM in *Streptomyces* as well as GM resistance in mycobacteria revealed the amplification the *dnaN* gene. This gene encodes DnaN, the DNA polymerase sliding clamp that anchors the DNA to DNA polymerase and thus confers high processivity to the replicative enzyme, resulting in the acceleration of the DNA replication and repair in prokaryotes.

Kling et al. (2015) demonstrate that DnaN is the molecular target of GM by performing surface plasmon resonance (SPR) analyses of GM, the methyl and cyclohexyl derivatives using the DnaN from different bacteria as well as human DnaN (i.e., PCNA). Whereas the dissociation constants could be measured for the bacterial DnaN with all GM derivatives, no binding to the human PCNA was detected, pointing to a good selectivity. The SPR results were further supported by X-ray structures of co-crystals of GM and CGM with DnaN. The GM derivatives bound to a hydrophobic pocket between domain II and III of the clamp, which was previously characterized as the interaction site between the sliding clamp and DNA polymerase as well as other DNA-modifying enzymes.

CGM is orally bioavailable, and mouse experiments performed by oral administration show that in an acute mouse model, the bacterial growth and gross lung lesion could be prevented applying a dose of 50 mg/kg of CGM. In a chronic model, the number of colony forming units could be decreased to the same extent as with rifampicin. These findings of monotherapy initiated the test of combinations of GM with classical anti-Tb drugs. The combination with rifampicin and pyrazinamide gave impressive results stressing the high in vivo activity against replicating Mtb in addition to bactericidal activity against non-replicating bacteria.

Peptidic as well as small molecule inhibitors of similar sliding clamps have recently been described for some Gram-positive and -negative bacteria (e.g., Georgescu et al., 2008; Kjelstrup et al., 2013; Wolff et al., 2014; Yin et al., 2014, 2015) emphasizing the significance of this target. However, none of these inhibitors is as potent as CGM and none is currently in clinical trials. Because there is no pre-existing resistance due to the new anti-Tb mechanism of action, and the frequency of resistance seems to be low, GM and its derivatives have the potential to be moved forward to preclinical trials.

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