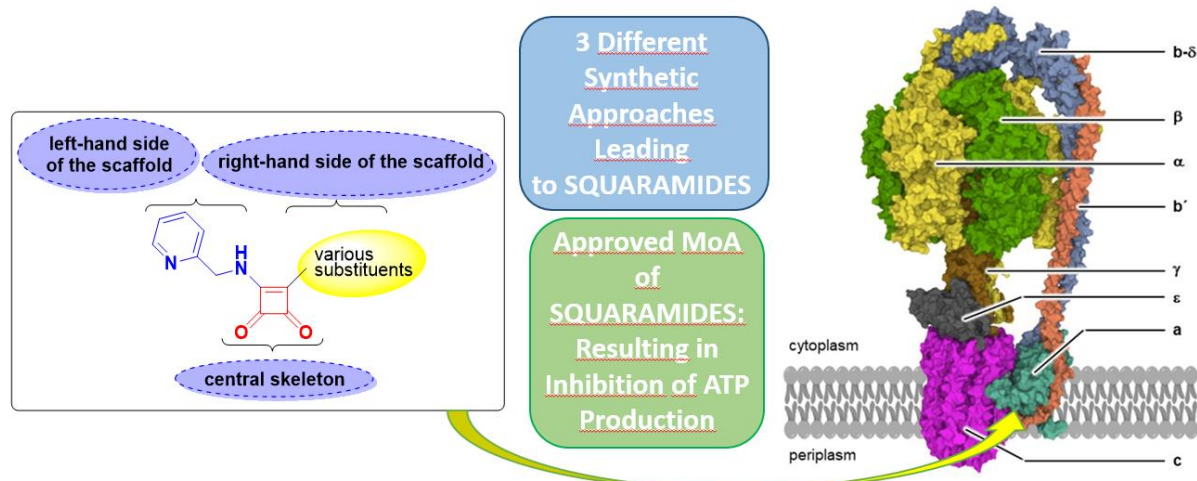


## Squaramides: New Antituberculosis Agents Effective Against BDQ-Resistant Strains

Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis*, still remains a serious health issue with global impact. Despite significant efforts in prevention, TB continues to pose a serious threat. Therefore, there is a pressing need for novel chemical scaffolds displaying potent antimycobacterial properties and novel modes of action. In response to this challenge, we focused our attention on squaramides as potential inhibitors of mycobacterial ATP synthase.

Over the years, we synthesized more than 60 squaramide derivatives and evaluated their *in vitro* biological activity. This broad compound library enabled us to systematically analyse the structural features contributing to their efficacy. Additionally, *in silico* testing allowed us to rationalize these structural insights. Notably, we successfully identified the binding site of squaramides on the mycobacterial ATP synthase. They specifically bind to subunit a of the mycobacterial enzyme (Fig. 1). This key discovery facilitated the interpretation of our computational assays, streamlining the process of designing potentially active molecules. Also crucial in this context was the discovery that the binding site of squaramides on the target enzyme differs from that of bedaquiline (BDQ), the first approved inhibitor of mycobacterial ATP synthase. Consequently, squaramides exhibit activity even against BDQ-resistant strains of *M. tuberculosis*.

Armed with this structural understanding, we were able to rationally design new series of compounds. However, the synthesis of these derivatives presented challenges due to their structural complexity and the necessity of introducing aromatic substituents crucial for their biological activity. Consequently, we suggested multiple synthetic strategies over time to achieve successful compound synthesis. Specifically, we employed different approaches involving Friedel-Crafts-type reactions, reaction with organolithium salts, and ultimately, Liebeskind-Srogl cross-coupling reaction to effectively introduce the desired aromatic substituents onto the squaramide scaffold.



**Figure 1:** Squaramides as new group of mycobacterial ATP synthase inhibitors.

### Bibliography:

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