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# Batumin does not exert its antistaphylococcal effect through inhibition of aminoacyl-tRNA synthetase enzymes

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Sir,

We write in response to an article regarding the antibiotic batumin by Klochko *et al.* that appeared earlier this year in the journal [1]. The stated aim of this study was "to identify possible molecular targets for batumin as well as mechanisms of its antistaphylococcal activity". Apparently on the basis that batumin and the clinically deployed antibacterial drug mupirocin share a 9-hydroxynonanoic acid moiety, and that the biosynthesis of both antibiotics is directed by operons that exhibit some degree of sequence similarity, the authors formulated the hypothesis that batumin shares the same molecular target as mupirocin: the isoleucyl-tRNA synthetase (IleRS) enzyme that plays an essential role in protein synthesis. With a view to providing support for this hypothesis, Klochko *et al*. undertook two *in silico* investigations. The first of these involved molecular docking of batumin into X-ray crystal structures of IleRS, the results of which implied that batumin and mupirocin might bind with similar affinity to this enzyme. The second entailed analysis of the genome sequence of a batumin producer organism (*P. batumici*), which led to the identification of three genes encoding paralogues of leucyl-tRNA synthetase (LeuRS), and prompted the authors to suggest that batumin might also/ predominantly target LeuRS.

Whilst *in silico* analyses such as these may have a place in generating or refining a hypothesis as to the mode of action (MOA) of batumin, the results they provide are predictions at best, and do not constitute evidence to support the hypothesis that batumin acts by inhibiting aminoacyl-tRNA synthetase (aaRS) enzymes. In consequence, and lacking a direct experiment to test their hypothesis, the study by Klochko *et al.* did not progress beyond pure speculation. Though the authors made mention of the fact that their ideas would ultimately require experimental corroboration, this did not prevent them from presenting firm conclusions regarding the MOA of batumin that went well beyond what their results could justify (e.g. "It was found that batumin acted very similarly to mupirocin by inhibiting aminoacyl tRNA synthetases."). Here we present experimental evidence to show that their conclusions regarding the MOA of batumin are in fact wrong.

Antibiotics that mediate their antibacterial effect through inhibition of aaRS enzymes will act to rapidly deplete the bacterial cell of charged tRNA species, an early consequence of which will be inhibition of protein synthesis. For example, when mupirocin at 4XMIC is added to logarithmic phase cultures of *Staphylococcus aureus* strain SH1000, a dramatic (~65%) reduction in protein synthesis is observed within 10 minutes relative to an untreated control, as determined [2] by measuring incorporation of the radiolabeled amino acid L-[3,4-$^{3}$H(N)]-glutamine into polypeptides (Figure 1). By contrast, in an otherwise identical experiment using batumin (Enzo Life Sciences, Exeter, UK) at 4XMIC in place of mupirocin, no inhibition of protein synthesis was observed (Figure 1). It is therefore apparent that the antibacterial action of batumin is distinct from that of mupirocin, and does not involve inhibition of one or more aaRS enzymes.

A prior hypothesis regarding the MOA of batumin considered that this compound exerts its antibacterial effect through direct inhibition of fatty acid biosynthesis [3]. This proposal stemmed from the observation that an isoform (BatG) of the fatty acid synthesis enzyme, FabI, is encoded within the batumin biosynthesis cluster in some producer strains, and that heterologous expression of *batG* in *Escherichia coli* and *S. aureus* confers a substantial reduction in batumin susceptibility [3]. With a view to reconciling their speculations with these observations, Klochko *et al*. proposed that both batumin and mupirocin mediate their antibacterial effect by indirectly impairing fatty acid synthesis as a secondary consequence of inhibiting aaRS enzymes and inducing the stringent response. We examined the effect of 4X MIC batumin on fatty acid synthesis in *S. aureus* SH1000 by measuring incorporation of the radiolabeled precursor, [1,2-$^{14}$C]-acetic acid. Batumin and a known inhibitor of this pathway, triclosan, both caused a dramatic reduction (>60%) in fatty acid synthesis relative to the untreated control in 10 minutes (Figure 1). That batumin achieved such rapid and substantial inhibition of fatty acid synthesis, and before any detectable impact on the synthesis of other cellular macromolecules (protein), corroborates the original hypothesis that batumin directly inhibits fatty acid synthesis. The proposal that aaRS inhibitors ultimately mediate their antibacterial effects through indirect inhibition of fatty acid synthesis is not supported by the

observation that mupirocin, though demonstrating considerable inhibition of protein synthesis, exerted no inhibitory effect on fatty acid synthesis (Figure 1).

Thus, whilst further experimental studies will be required to more precisely delineate the MOA of batumin, the available evidence discounts inhibition of one or more aaRS enzymes, and implies that batumin mediates its antibacterial effect directly through inhibition of fatty acid biosynthesis.

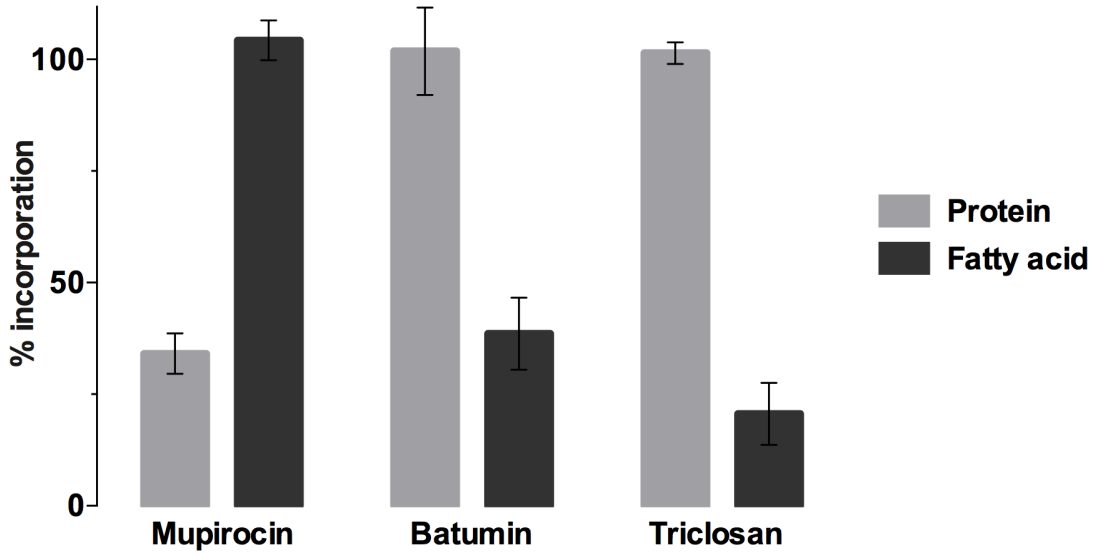


**Figure 1. Effect of batumin and control agents at 4XMIC on protein and fatty acid synthesis in *S. aureus* SH1000, as measured by incorporation of radiolabeled precursors over a 10 minute period.** Incorporation is shown as a percentage of that in the untreated control. Datum points represent the means of at least three experimental determinations, and error bars show standard deviations.

## References

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