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NEW CHEMICALS FOR FLYSTRIKE CONTROL

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SUMMARY
The project aimed to address the need for new chemicals for the control of the sheep blowfly by exploring the potential of a new class of insecticides. We designed and synthesised experimental compounds and showed that they were very effective at killing blowfly larvae, both in the laboratory and on sheep, at concentrations comparable to the commercial blowfly insecticide cyromazine. Further work to build on the outcomes of the present study to develop these insecticides for blowfly control will require the project team engaging with animal health companies. Given the ability of the blowfly to develop resistance to the chemicals used for its control, it is important that new classes of insecticides, such as the type explored in the present project, are in the development pipeline to provide these future chemical control options.

PROJECT REPORT
Control of the sheep blowfly relies largely on the use of chemical insecticides applied as preventative treatments to protect against flystrike. However, recent reports of the emergence of resistance to the most commonly-used chemicals threaten the sustainability of the industry, and have highlighted the need for alternative drugs for flystrike control. The present project aimed to explore one avenue of this drug development process by examining the potential for blowfly control based on the use of inhibitors of a specific target in the blowfly. The target was a group of enzymes that play a vital role in cell development in most organisms, histone deacetylase enzymes (HDACs). In recent years there has been a great deal of interest in developing inhibitors of these enzymes in humans as possible treatments for cancers, inflammatory diseases, and parasitic diseases. The present project aimed to identify inhibitors of HDAC enzymes for use as insecticidal compounds for the control of the sheep blowfly.

Experimental HDAC inhibitors were synthesised, and their ability to kill blowfly larvae was measured using *in vitro* assays. We also measured the ability of the compounds to inhibit the blowfly HDAC enzymes. We undertook repeated rounds of compound synthesis and testing, using the results of each round to inform on structural changes to be made to compounds for the next round of synthesis. We also performed a comprehensive homology modelling study to generate likely structures of the blowfly HDAC enzymes. This then allowed us to model the fit of experimental drugs into the enzymes. The homology modelling also allowed us to study differences that exist between the structures of the enzymes in blowflies and mammals, with a view to exploring the potential for drug design of blowfly-specific inhibitors. Finally, to begin to translate our study from the lab to the field, we conducted a small scale larval-implant trial on sheep using several of our experimental compounds.

We examined the ability of blowfly larvae to establish strikes on sheep at sites that have been treated with the experimental drugs.

The most potent compounds identified in the study had very significant levels of activity against blowfly larvae *in vitro*, and were also potent inhibitors of blowfly HDAC enzymes. The best of the compounds was within 4-fold as toxic to blowfly larvae as the commercial blowfly control chemical cyromazine (the active ingredient in Vetrazin, ProGuard, Lucifly and Cy-Guard) in our *in vitro* assays. Importantly, the most potent compounds showed an ability to inhibit the early larval life stages of the blowfly, with complete inhibition of larval growth within the first 24 hours at the highest concentrations tested. This speed of action of the compounds is an important aspect for their potential as insecticides as it is vital for a blowfly control chemical to prevent the larvae developing to a stage that can start to cause significant damage to the sheep.

We constructed *in-silico* homology models for each of the five blowfly HDAC proteins *Lc*HDAC1, 3, 4, 6 and 11, as identified in its genome. The various blowfly HDACs had between 44 -78% sequence identity with their respective human HDACs (1, 3, 4, 6, 11). We analysed the amino acid differences between the blowfly HDACs and their corresponding human HDACs near the binding site. We found the binding sites of three of the blowfly enzymes were very similar to human binding sites with few differences. Hence, the design of inhibitors that are selective for these blowfly enzymes over their human counterparts will be challenging. On the other hand, for another of the blowfly enzymes (*Lc*HDAC6), we found significant sequence differences between the human and blowfly binding sites. Drug docking studies confirmed the presence of a number of differences near the binding site of the human and blowfly HDAC6 enzymes. These differences in residue size, charge and polarity may allow the design of new inhibitors that may prove to be more potent and selective towards blowfly HDACs.

Finally, we conducted a sheep trial in which experimental compounds were applied to sites on sheep, and the subsequent ability of blowfly larvae to establish infections at these sites was measured (all animal procedures were approved by the CSIRO Armidale Animal Ethics Committee, approval number 19/04). We applied cyromazine (ProGuard) to some sites as a commercial insecticide treatment to compare to our experimental compounds. We tested three experimental inhibitors, chosen on the basis of potency against blowfly larvae *in vitro*, high microsomal metabolic stability, presence of structurally-distinct features, and low synthetic cost. Two of the three compounds killed all the larvae at the experimental sites. The level of drug required to kill all larvae was approximately 5-fold higher than the levels of cyromazine required to achieve the same outcome. This indicates that the experimental compounds were able to prevent blowfly larval growth at a concentration similar to that for the commercial product cyromazine.

Figure 1. Measuring the ability of experimental compounds to prevent growth of blowfly larvae on sheep. Left: experimental sites on a sheep; Right: addition of freshly-hatched blowfly larvae on a paper disc to a drug-treated experimental site.

We investigated whether any of the experimental drugs were blowfly-specific inhibitors (that is, inhibitors of blowfly HDAC enzymes, but not of mammalian HDAC enzymes), but found no evidence for this. However, as mentioned above, the homology modelling work showed that a focus on the *Lc*HDAC6 enzyme offers potential for the discovery of such blowfly-specific inhibitors in the future. It is also clear that complete insect-specificity may not be required for blowfly control as the potency of the experimental compounds identified here means that they can likely be used at levels safe for topical application to mammals, as required for blowfly control in sheep.

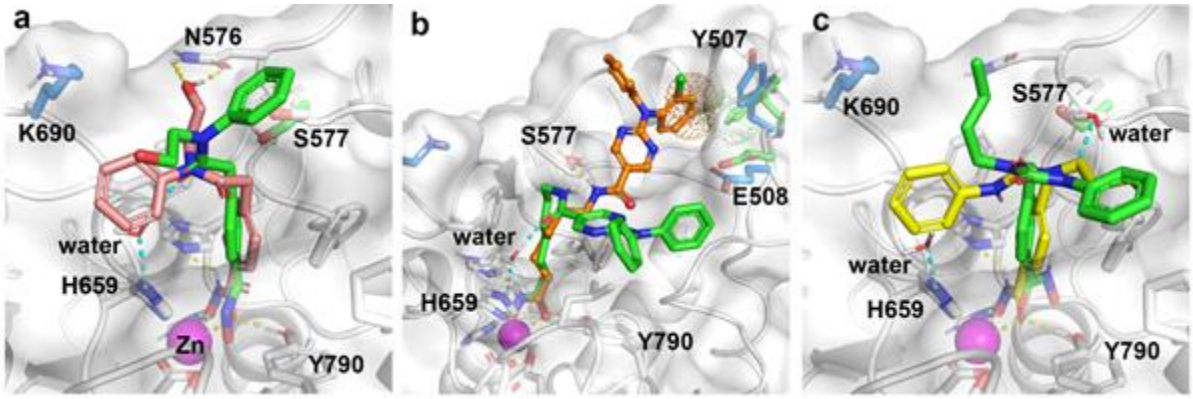

Figure 2. Modelling of blowfly HDAC enzymes to determine how well experimental compounds 'fit' into their target enzyme in the blowfly.

This project has shown that HDAC inhibitors are potent inhibitors of blowfly larval growth and development *in vitro* and has identified new potent compounds. We have shown that the most potent compounds are also able to prevent the development of larvae at experimental sites on sheep. The project represents the first stage of a process in drug development that can take many years. It would be at least 5 years before any insecticide based on inhibition of HDAC enzymes could be delivered to the market, perhaps longer. In the meantime, the wool industry is able to utilise the currently available insecticides, with the knowledge however that resistance to the dicyclanil-based products is emerging. If resistance to this chemical becomes more wide-spread, and resistance also emerges to the currently-used ivermectin- and imidacloprid-based products, the availability of new insecticides will become more important and their rapid development more urgent. It is therefore important that new classes of insecticides, such as the type explored in the present project, are in the development pipeline to provide these future chemical control options.

Further work to build on the outcomes of the present study to develop HDAC inhibitors as insecticides for blowfly control will require the project team engaging with animal health companies.

FURTHER INFORMATION

A final report on this project is available at www.wool.com/flystrikelatest.

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