Towards the Commercialisation of New Environmentally Friendly Insecticides, including those Targeting Sheep Body Louse and Blowfly Ecdysone Receptors

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Executive Summary

A class of insecticides targeting ecdysone receptors and selective for the order Lepidoptera was in a random screen of molecules against whole insects. These insecticides are so safe for man and other insects that they have received a Presidential Green Chemistry award, are used as insecticides on the one hand and as pharmaceuticals for the control of therapeutic genes in humans on the other. Our studies on the hormone binding domains of ecdysone receptor heterodimeric (EcR-USP) targets of these molecules have provided insights into the basis of their selectivity for insect orders at a molecular level. We have also developed an automated high throughput assay to detect new molecules that bind to these receptors.

The overall aim of this project was to screen a selected DuPont library of 5000 different molecules against ecdysone receptors from the sheep ectoparasites *Lucilia cuprina* and *Bovicola ovis* for compounds binding to the receptors and therefore potential insecticidally compounds. To this end, functional recombinant ecdysone receptor ligand (hormone and insecticide) binding domain (LBD) proteins from the two parasites were produced in cultured insect cells and purified.

The purified proteins were then employed to arm our novel automated fluorescence polarisation assay to screen the DuPont library for receptor binding compounds. Some 240 confirmed hits were found for the *L. cuprina* ecdysone receptor and 120 for the *B. ovis* receptor.

A high-resolution X-ray atomic structure (2.2 Å) was also obtained for the *B. ovis* ecdysone receptor target and progress made towards the corresponding structure from *L. cuprina*. The X-ray structure of the *B. ovis* ecdysone receptor LBD is available to guide optimisation potential insecticidal of chemistries by medicinal chemistry.

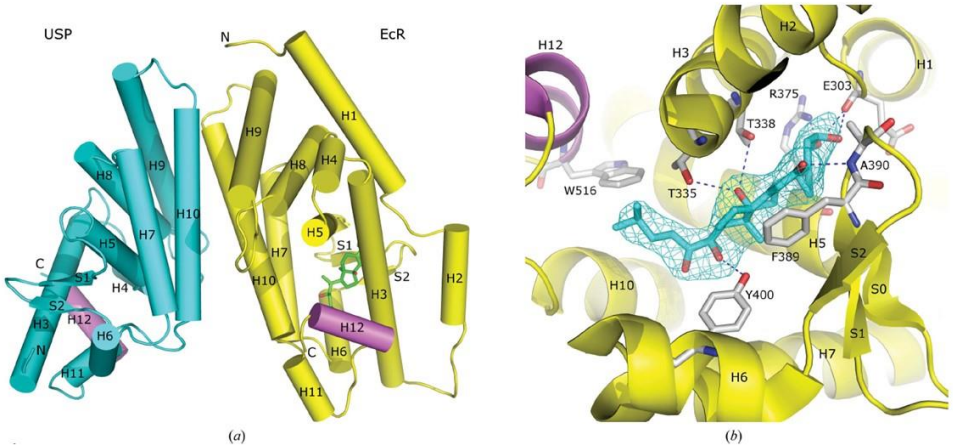

Figure 1. X-ray structure of (a) the ligand binding domain of the B. ovis ecdysone receptor hormone/insecticide binding domain and (b) higher magnification of the hormone binding pocket containing an ecdysteroid hormone (cyan).

On the strength of these results, DuPont awarded an additional grant (matched by AWI) for *in vivo* testing of a promising subset of the compounds to induce mortality in the sheep ectoparasites. Twenty of the strongest ecdysone receptor-binding compounds were selected for *in vivo* testing. One of these at 10 ppm lowered the emergence of adult blowflies by 70%. At 100 ppm six compounds caused 175-100% lethality of *B. ovis*. At 10 ppm one of these caused 100% lethality and another 72% lethality.

This project has validated the use of our novel automated ecdysone receptor-based screen to discover new chemistries that kill sheep ectoparasites, provided a high-resolution atomic structure of the receptor target to guide optimization of these molecules and furthermore has supplied promising lead compounds for optimization by medicinal chemistry to advance commercial development.

AWI invests in research, development, innovation and marketing activities along the global supply chain for Australian wool. AWI is grateful for its funding which is primarily provided by Australian woolgrowers through a wool levy and by the Australian Government which provides a matching contribution for eligible R&D activities