Australian Wool Innovation Limited

2018 BREECH FLYSTRIKE RD&E

TECHNICAL UPDATE

New Chemicals for Blowfly Control

Andrew Kotze – CSIRO

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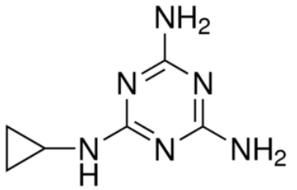
The need for new chemicals

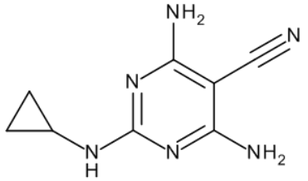
- Limited number of drugs for protection against strike
- Low level *in vitro* cyromazine resistance reported in 2012,
  also, cross-resistance to dicyclanil

  products remain effective against field populations



cyromazine

- But, clear change in fly response,
  below level of impact in field



dicyclanil

- *In vitro* cyromazine resistance now ‘quite common’
- Ivermectin resistance common in other insect species

Highlights the need to develop new chemical controls
- unrelated to cyr. / dicycl. or ivermectin

Histone deacetylases (HDACs) as drug targets

- normal cell:

DNA → RNA → protein → cell growth

if add HDAC inhibitors: normal cell growth escalates out of control

DNA → RNA → protein → 

- HDAC inhibitors used in cancer therapy
- Under investigation as anti-parasitic agents
  - malaria
  - blood-flukes
  - leishmania
- Blowfly genome revealed sequences of HDACs in the blowfly is species

Potential of HDAC inhibitors as insecticides ??

Project objectives

Collaboration with Prof. David Fairlie (Inst. Molecular Biosciences, Uni of QLD)
- HDAC expertise and experimental HDAC inhibitors

- Bioassays (whole maggot assays): - to identify potent insecticidal compounds
  - structure / activity relationships

- Molecular: - clone *Lucilia* HDACs
  - recombinant expression
  - high-throughput enzyme assays
- Homology modelling: to inform on structural requirements for inhibitors
- Compounds to test: commercial suppliers and synthesis in Fairlie lab
- Seek animal health company investment
  blowfly as model for wider insecticide use

Insect selectivity will be crucial

We are not dealing with an insect-specific target

- malaria and blood-fluke research more advanced
  → provides some optimism for selectivity

- Catalytic domain amino acid comparisons;
  human *vs* blowfly, percent different:

HDAC1	14 %
HDAC3	27%
HDAC4	39 %
HDAC6	53 %
HDAC11	44 %

Significant differences between blowfly and human

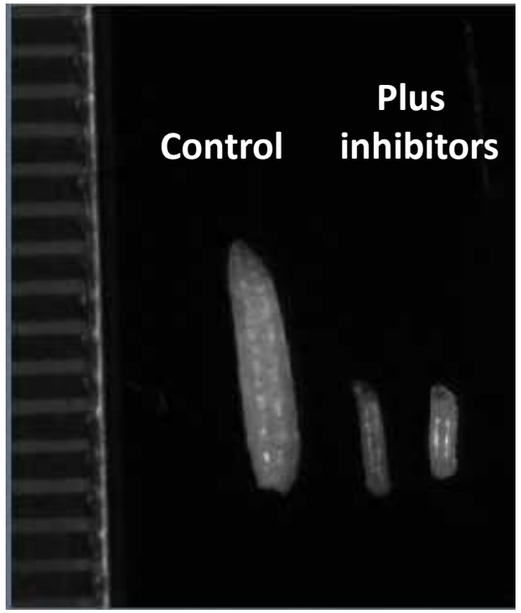
Good for insect-selectivity

- Recent advances in selectivity of inhibitors for human HDAC isoforms

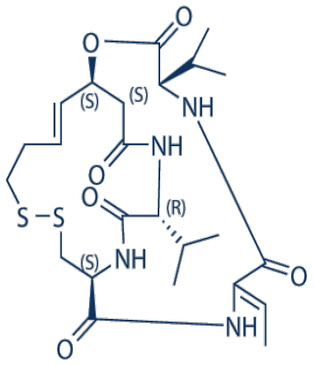
- We will test compounds against mammalian HDAC enzymes and cells

Maggot bioassays

HDAC inhibitors have significant effects on growth of sheep blowfly larvae



Example: Romidepsin



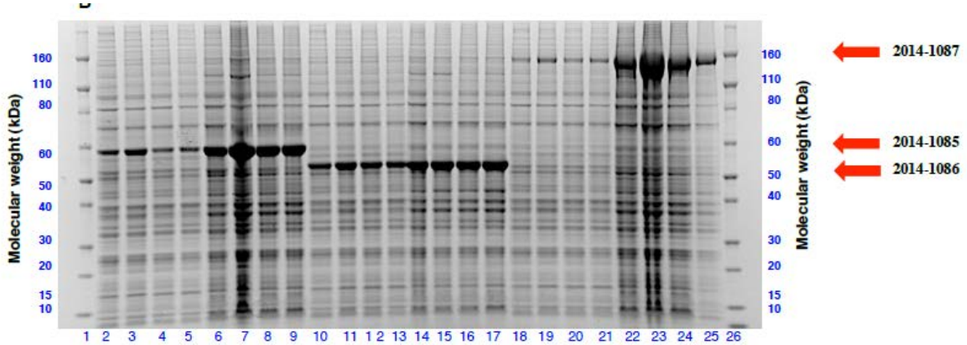
- More toxic to maggots than cyromazine
- Similar toxicity to dicyclanil

- BUT:
  - non-specific
  - complex structure

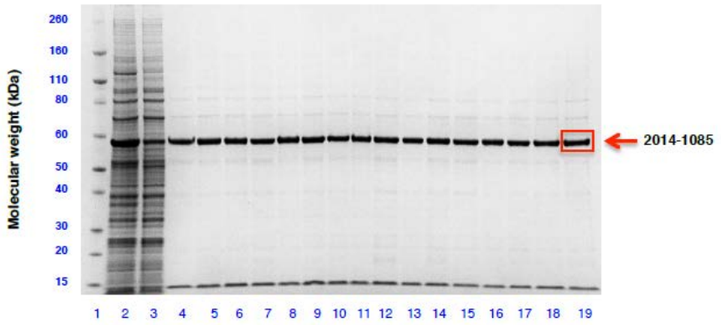
Recombinant enzyme expression

- Need to prepare pure blowfly HDAC enzymes
- Will use the enzyme in assays to study inhibitory effects of compounds
- Protein Expression Facility at Uni of QLD

Protein expression and purification



Blowfly HDAC1



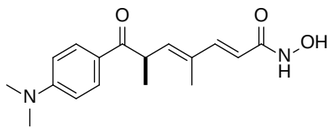
Have generated large amounts of HDAC 1 and 6;

Currently working on HDAC11

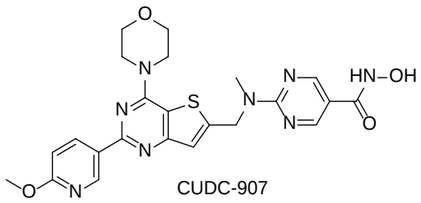
= representatives of the major enzyme classes in the blowfly

Identifying structures with potent activity

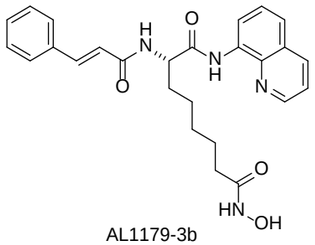
Hydroxamic acids:



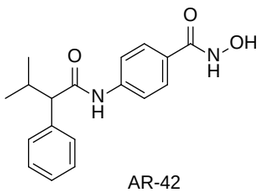
Trichostatin



CUDC-907

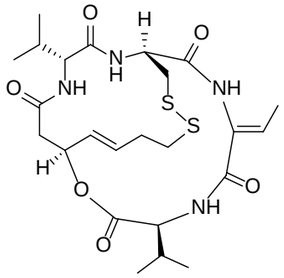


AL1179-3b



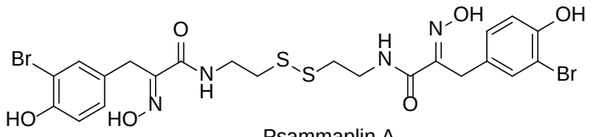
AR-42

Cyclic depsipeptide:



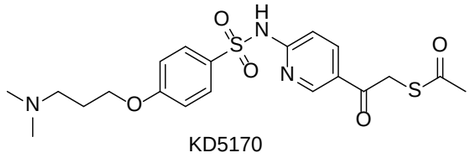
Romidepsin

Thiolate:



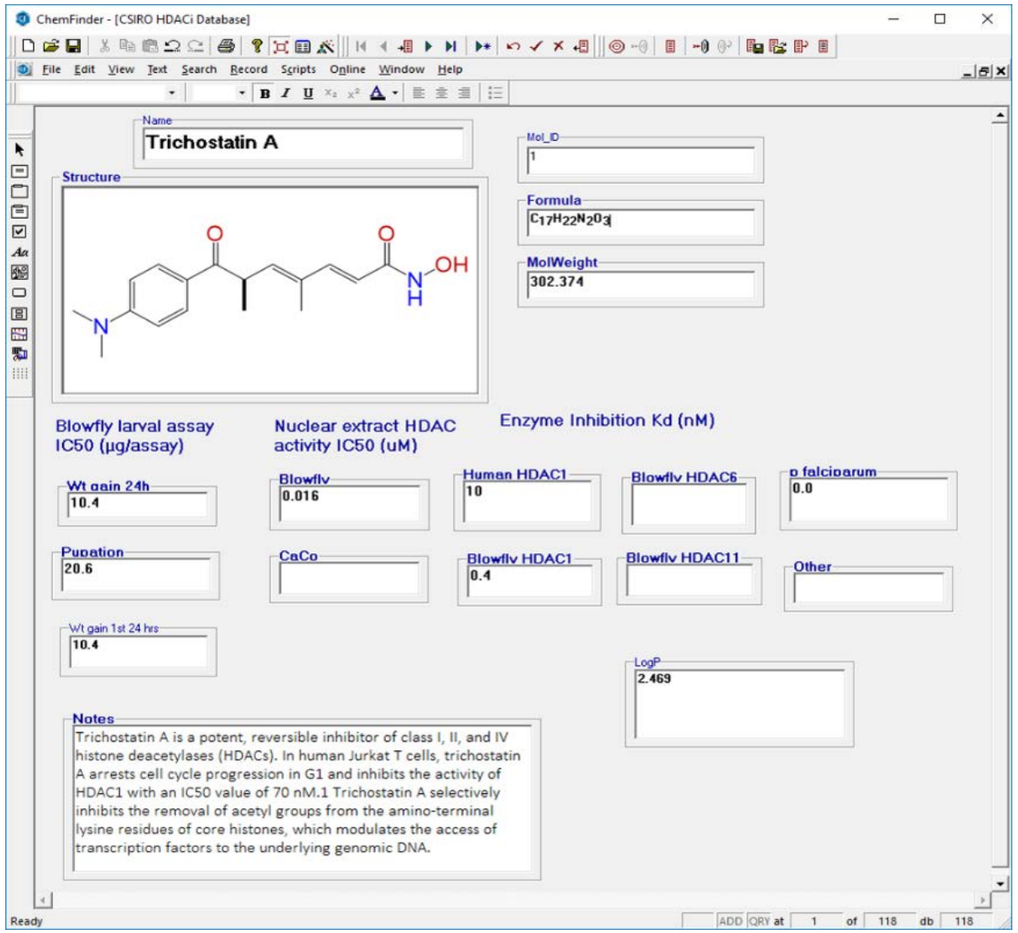
Psammaplin A

Thioester:

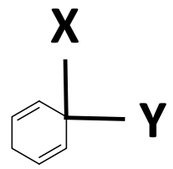
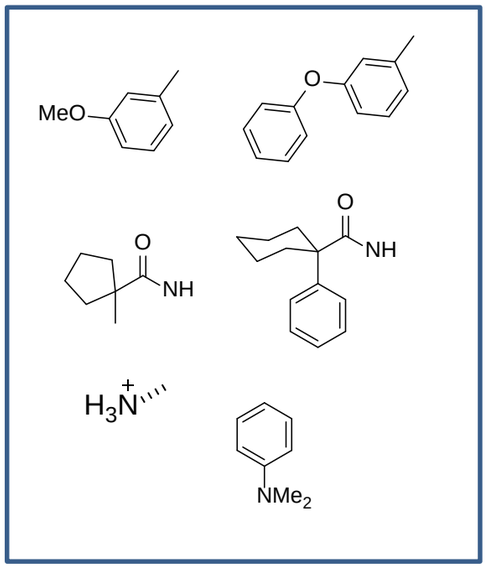
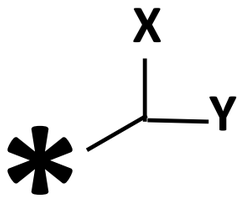
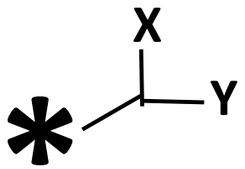
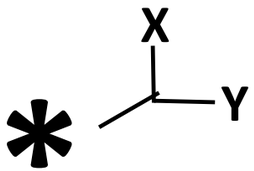


KD5170

Structure / activity database:



An example of the structure / activity process

Compound	Effect on blowfly growth - IC_{50} µg
Parent compound 	**32**
 	**63** Less active than parent
	**16** 2-fold better than parent
	**5** 6-fold better than parent

Control Treated

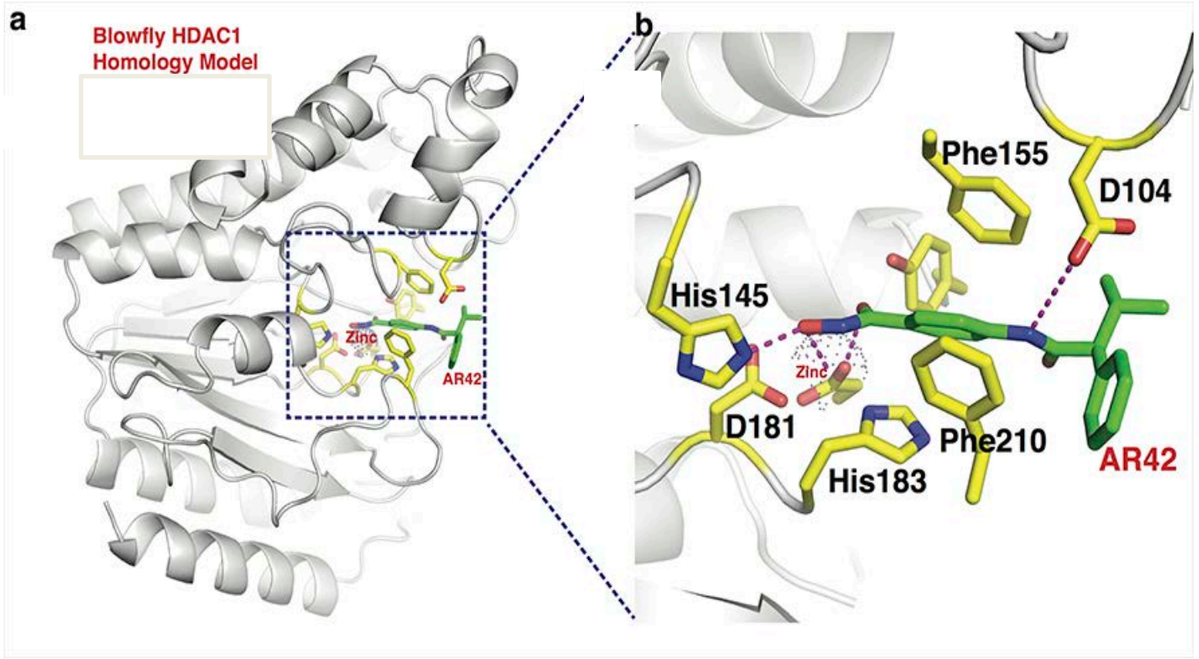
24hr

48hr

Homology modelling

David Fairlie, Wei Xu IMB

Blowfly HDAC1 homology model



Measure binding strength of inhibitors

- Can predict active chemical structures - binding strengths
- use this information to <u>design new compounds</u>
- Can compare blowfly and human models to look for <u>insect specificity</u>

Present position of project

Blowfly bioassays: HDAC inhibitors are very toxic towards blowfly larvae;
structure / activity studies ongoing
→ more potent compounds

Recombinant enzymes: have produced blowfly enzymes
can measure effects of all compounds on enzyme activity

Homology modelling: starting to design new compounds

Insect specificity: - measuring toxicity of most-active compounds on mammalian cells
- homology modelling

↓

Bioassay, enzyme and modelling tools in place to identify lead candidates as blowfly insecticides

Pathway to commercialisation

- AWI funding basic research:
  - target characterisation
  - discovery of lead compounds
- We will need to engage with an AgVet chemical company
- Blowfly market not big enough, therefore need wider <u>insecticide</u> role
  → using blowfly as proof-of-concept
- Time frame: 5—10 years
- We have started to talk to several animal health companies

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