

2018 BREECH FLYSTRIKE RD&E TECHNICAL UPDATE

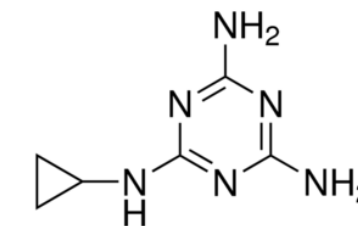
New Chemicals for
Blowfly Control

Andrew Kotze – CSIRO
17 July 2018

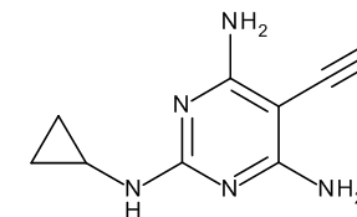


The need for new chemicals

- 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
- But, clear change in fly response, below level of impact in field
- *In vitro* cyromazine resistance now 'quite common'
- Ivermectin resistance common in other insect species




cyromazine



dicyclanil



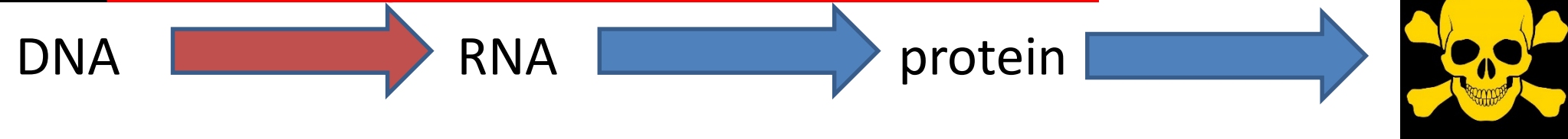
 **Highlights the need to develop new chemical controls**
- unrelated to cyr. / dicycl. or ivermectin

Histone deacetylases (HDACs) as drug targets

- normal cell:

DNA → RNA → protein → cell growth

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



- 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;

<u>human vs blowfly</u> , percent different:	HDAC1	14 %
	HDAC3	27%
	HDAC4	39 %
	HDAC6	53 %
	HDAC11	44 %

**Significant differences
between blowfly and human**

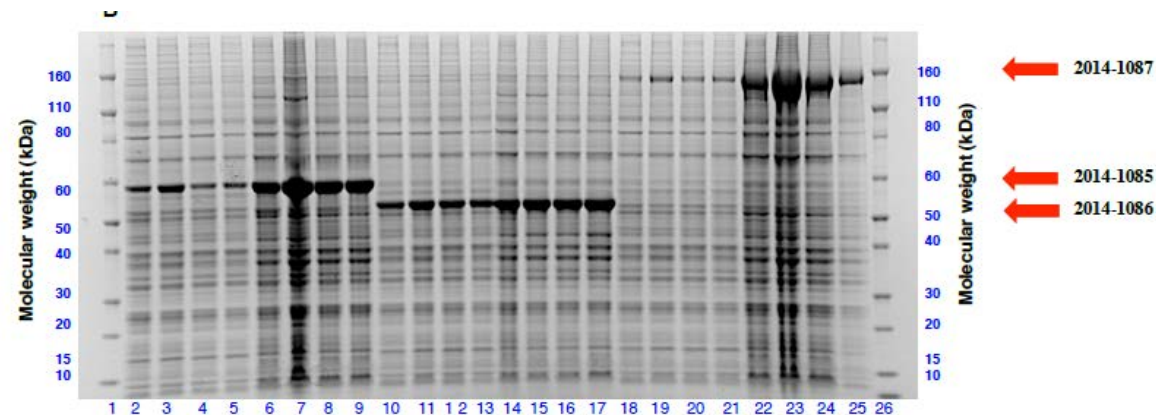
Good for insect-selectivity

- Recent advances in selectivity of inhibitors for human HDAC isoforms
- We will test compounds against mammalian HDAC enzymes and cells

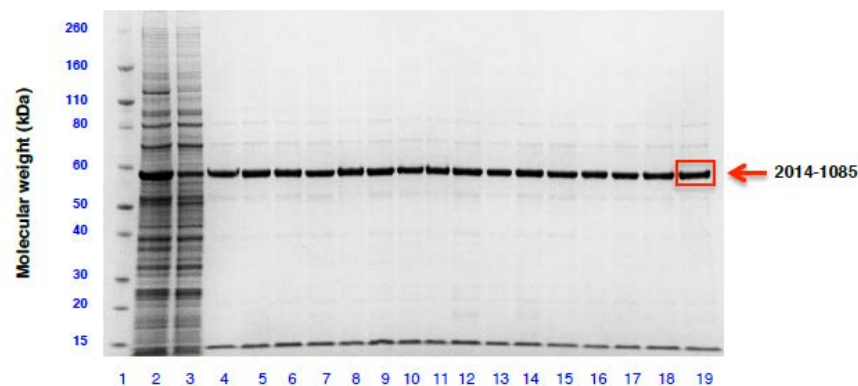
Recombinant enzyme expression

- 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;

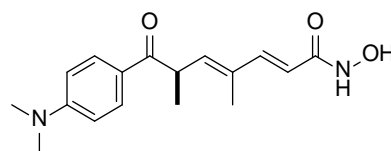
Currently working on HDAC11

= representatives of the major
enzyme classes in the blowfly

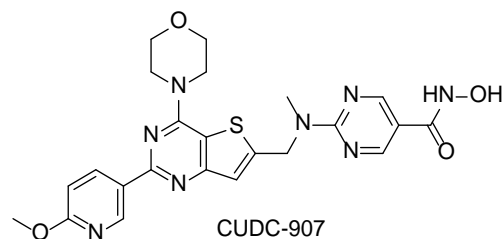
Identifying structures with potent activity

Structure / activity database:

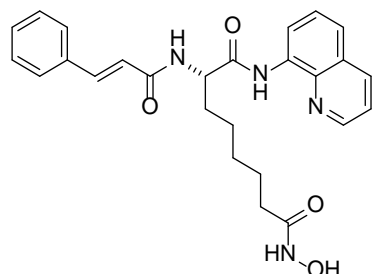
Hydroxamic acids:



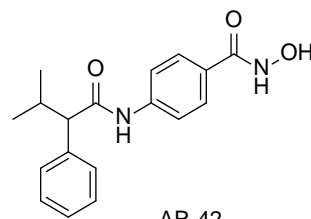
Trichostatin



CUDC-907

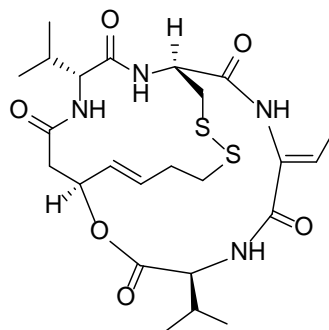


AL1179-3b



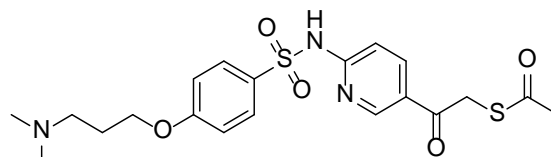
AR-42

Cyclic depsipeptide:



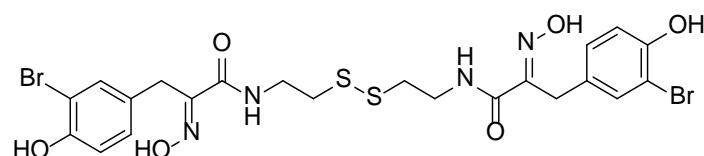
Romidepsin

Thioester:

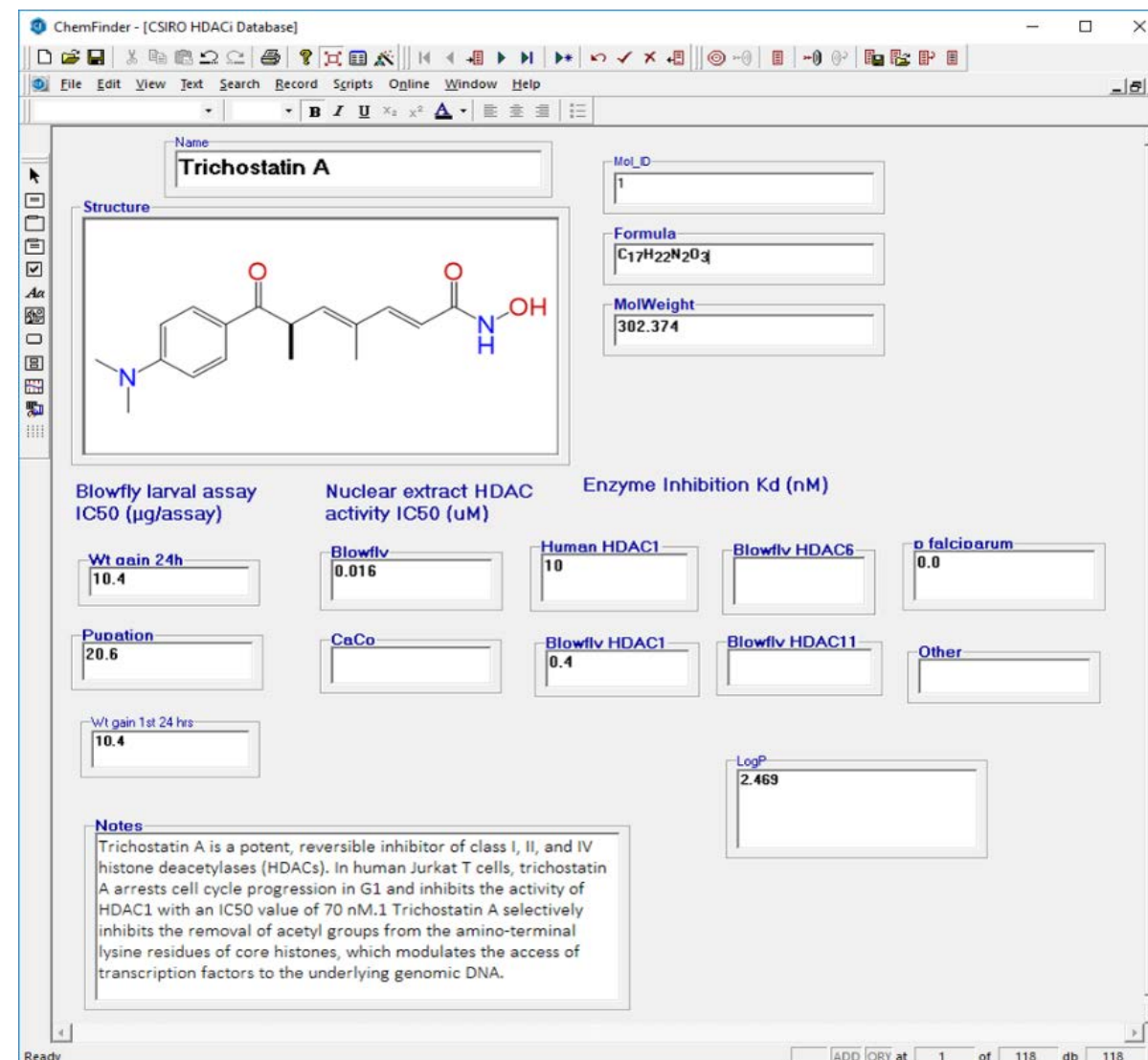


KD5170

Thiolate:



Psammaplin A



ChemFinder - [CSIRO HDACi Database]

Name: **Trichostatin A**

Mol_ID: 1

Formula: C₁₇H₂₂N₂O₃

MolWeight: 302.374

Blowfly larval assay IC50 (µg/assay)	Nuclear extract HDAC activity IC50 (µM)	Enzyme Inhibition Kd (nM)		
Wt gain 24h: 10.4	Blowfly: 0.016	Human HDAC1: 10	Blowfly HDAC6:	D. falciparum: 0.0
Purification: 20.6	CaCo:	Blowfly HDAC1: 0.4	Blowfly HDAC11:	Other:
Wt gain 1st 24 hrs: 10.4				LogP: 2.469

Notes: Trichostatin A is a potent, reversible inhibitor of class I, II, and IV histone deacetylases (HDACs). In human Jurkat T cells, trichostatin A arrests cell cycle progression in G1 and inhibits the activity of HDAC1 with an IC50 value of 70 nM. Trichostatin A selectively inhibits the removal of acetyl groups from the amino-terminal lysine residues of core histones, which modulates the access of transcription factors to the underlying genomic DNA.

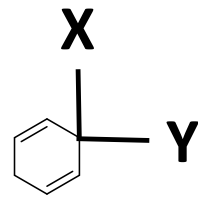
ADD QRY at 1 of 118 db 118

An example of the structure / activity process

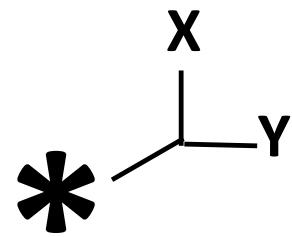
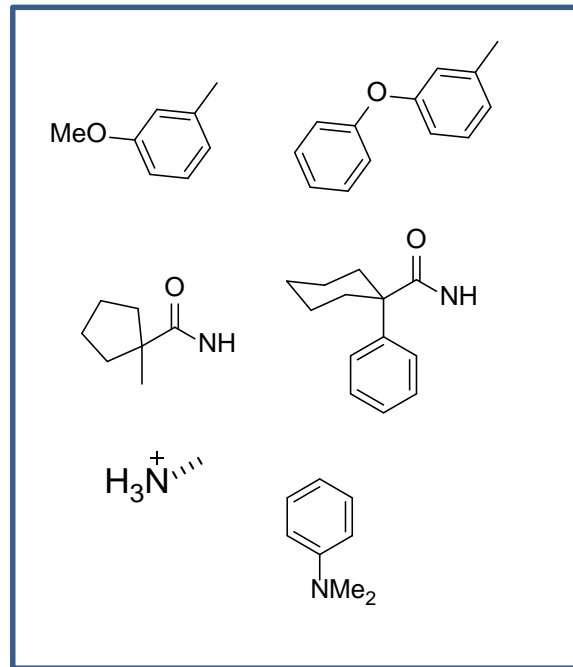
Compound

Effect on blowfly growth - IC₅₀ μg

Parent compound

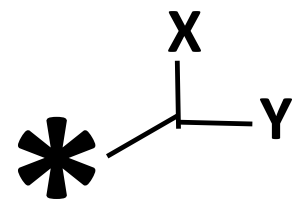


32



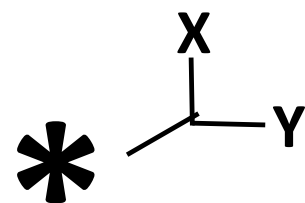
63

Less active than parent



16

2-fold better than parent



5

6-fold better than parent

Control

Treated

24hr

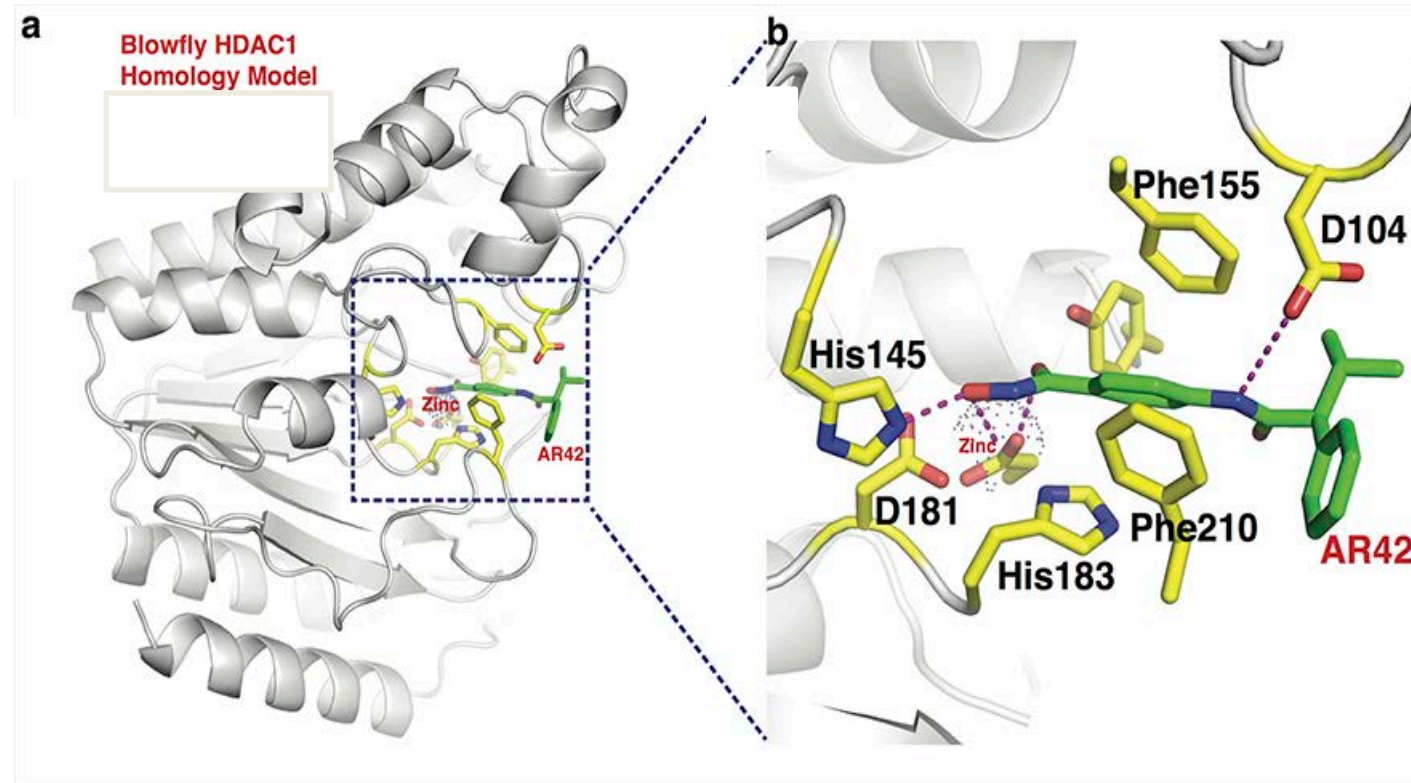
48hr



Homology modelling

Blowfly HDAC1 homology model

David Fairlie, Wei Xu IMB



Measure binding strength of inhibitors

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

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 insecticide role
 using blowfly as proof-of-concept
- Time frame: 5—10 years
- We have started to talk to several animal health companies

Acknowledgements



CSIRO: Neil Bagnall
Angela Ruffell



University of Queensland: Prof. David Fairlie
Bob Reid



**THE UNIVERSITY
OF QUEENSLAND**
AUSTRALIA

Post doc: Wei Zu

PhD students: Lilong Dong
Jaihui Tong
Darren Do

Funding: AWI





This publication is based on information presented at the Australian Wool Innovation Limited (AWI) National Wool Research and Development Technical Update on Breech Flystrike Prevention held on 17th July 2018. Some information in this publication has been contributed by one or more third parties and licenced to AWI, and AWI has not verified whether this information is correct. This publication should only be used as a general aid and is not a substitute for specific advice. Any reliance on the information contained in this publication is done at your own risk and to the extent permitted by law, AWI and any third party contributors exclude all liability for loss or damage arising from the use of the information in this publication. Except to the extent permitted under Copyright Law no part of this publication may be reproduced by any process, electronic or otherwise without the specific written permission of AWI. Neither may information be stored electronically in any form whatsoever without such permission. AWI gratefully acknowledges the funds provided by the Australian government to support research, development and marketing of Australian wool. GD2792