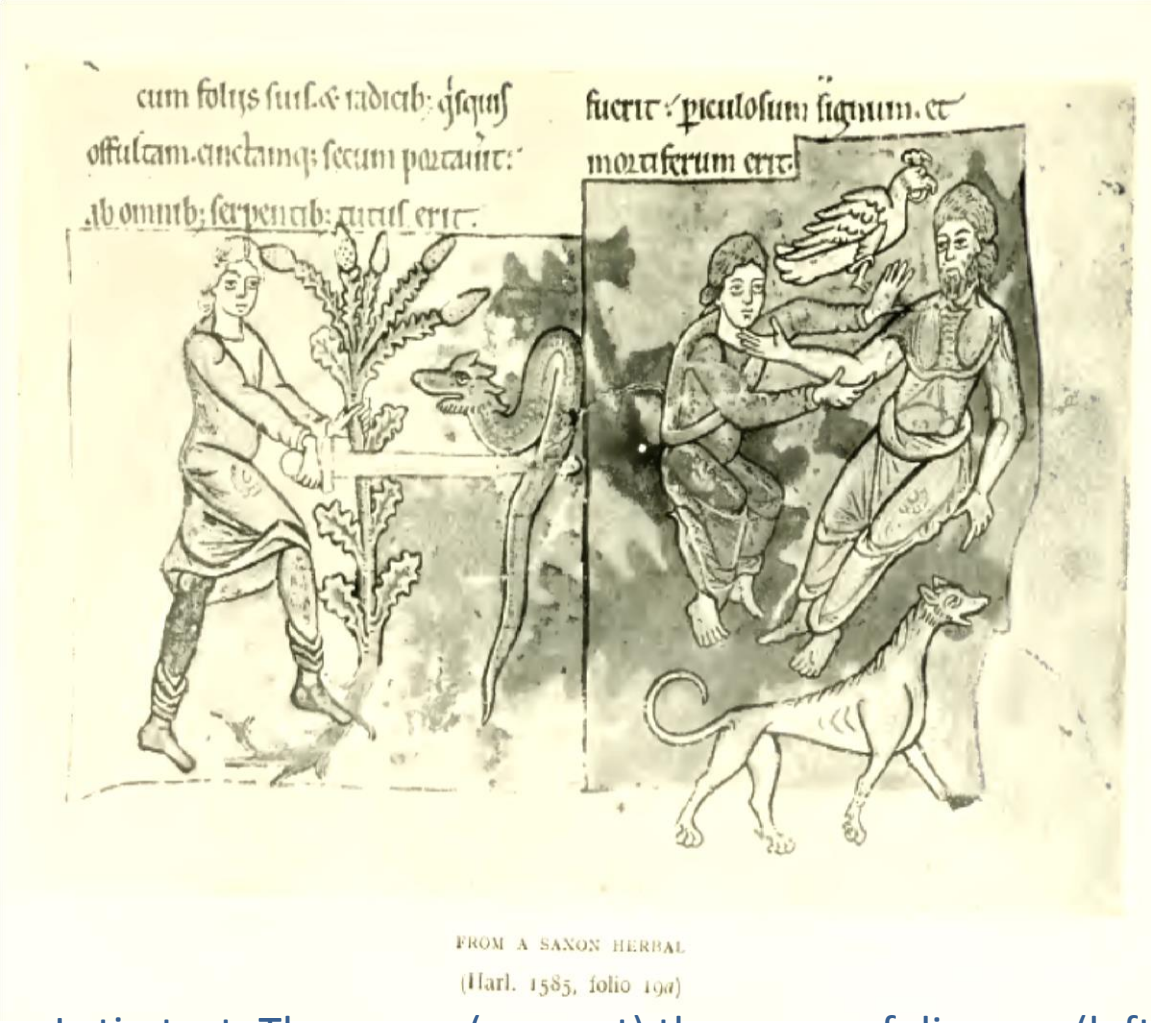
University of Strathclyde Science

Drug Discovery ‘Backwards’

Colin Suckling
University of Strathclyde,
Glasgow, Scotland

WestCHEM

Old English view: Disease-causing agents



FROM A SAXON HERBAL

(Harl. 1585, folio 19d)

Latin text. The worm (serpent) the cause of disease. (left)

Medicinal plants and medicines

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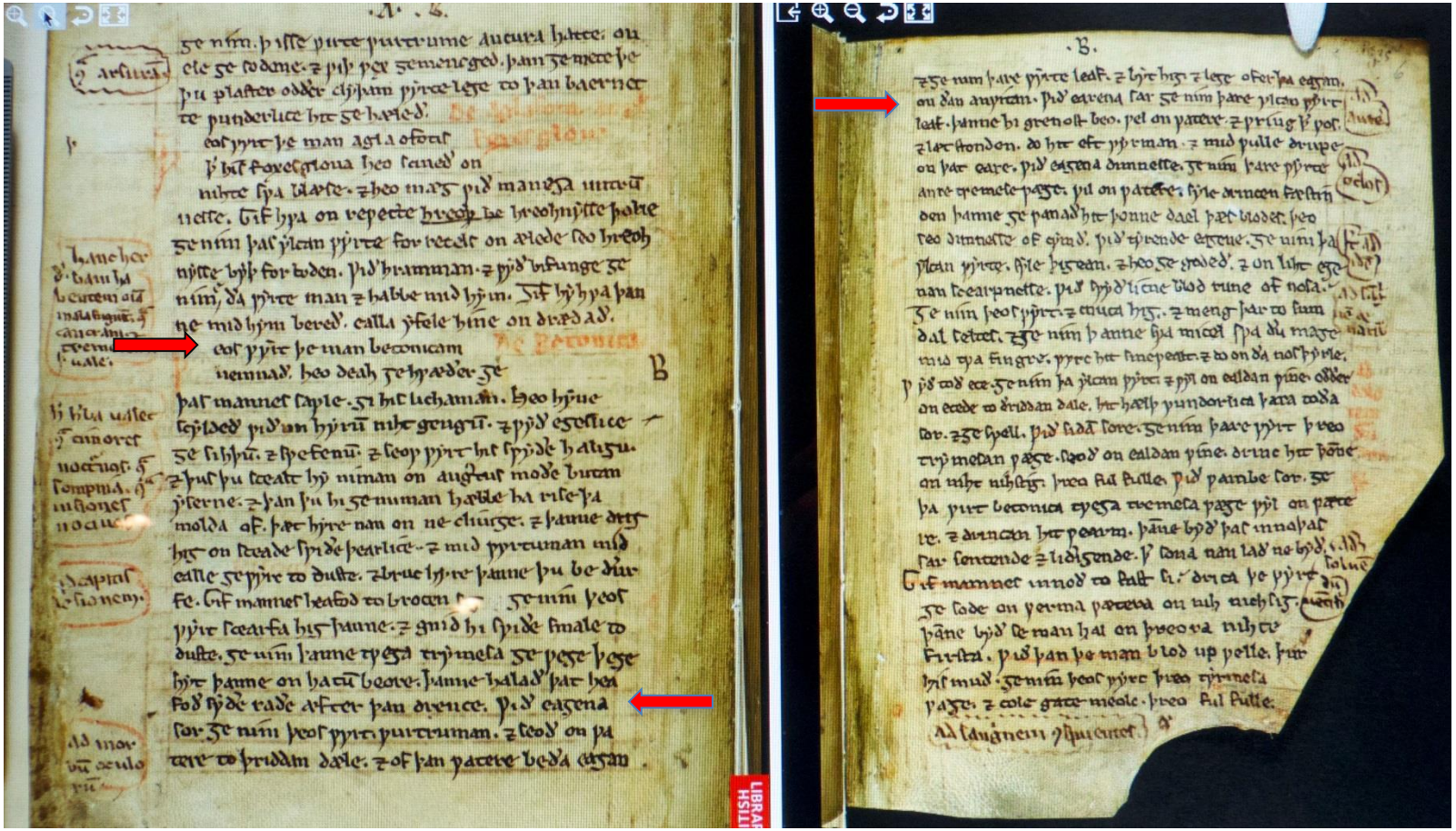

In translation

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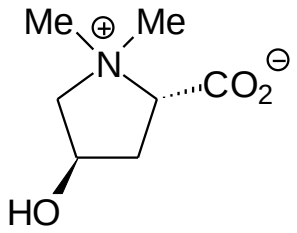
"This weed that is called Betony is found on meadows and on wild downs and in places of peace… It protects you against frightful prowlers in the night and against evil visions and dreams…

"To treat sore eyes, take the root of this weed, boil with three parts of water, and bathe the eyes with the water. And take the leaf of the same plant and place it over the eyes and the face."

"To treat sore ears, take the greenest part of the leaf of the same weed, boil in water and strain off the juice. While it is still warm, drip it into the ear through wool."

***Stachys officinalis* (L.) Trevis.,**
betony - alkaloid betonicine
Widely reported to have anti-inflammatory activity.
See also US website 'WebMD'



A Scottish contribution: 18th C

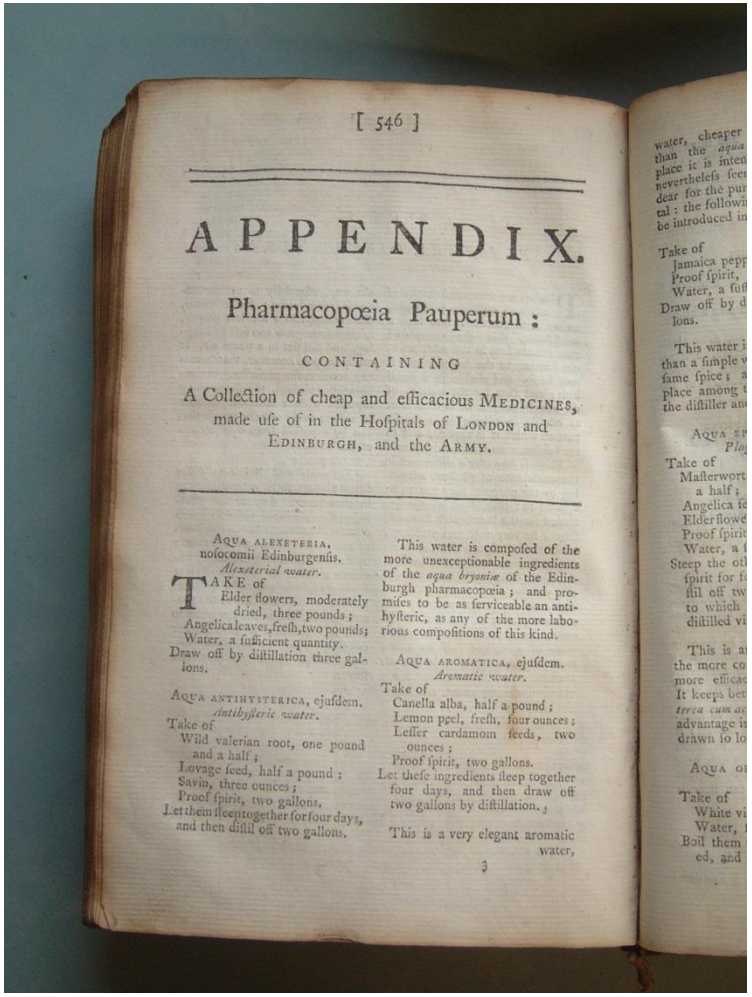
Pharmacopoeia Pauperum –

A collection of cheap and efficacious medicines.

Aqua Antihysterica Edinburgensis

Several herbs

Main ingredient – 2 gallons proof spirit!



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APPENDIX.

Pharmacopœia Pauperum:

CONTAINING

A Collection of cheap and efficacious MEDICINES, made uſe of in the Hoſpitals of LONDON and EDINBURGH, and the ARMY.

AQUA ALEXETERIA, noſocomii Edinburgenſis. *Alexeterial water.*

TAKE of
Elder flowers, moderately dried, three pounds;
Angelica leaves, freſh, two pounds;
Water, a ſufficient quantity.
Draw off by diſtillation three gallons.

This water is compoſed of the more unexceptionable ingredients of the *aqua bryoniæ* of the Edinburgh pharmacopœia; and promiſes to be as ſerviceable an antihyſteric, as any of the more laborious compoſitions of this kind.

AQUA ANTIHYSTERICA, ejuſdem. *Antihyſteric water.*

Take of
Wild valerian root, one pound and a half;
Lovage ſeed, half a pound;
Savin, three ounces;
Proof ſpirit, two gallons.
Let them ſteep together for four days, and then diſtil off two gallons.

AQUA AROMATICA, ejuſdem. *Aromatic water.*

Take of
Canella alba, half a pound;
Lemon peel, freſh, four ounces;
Leſſer cardamom ſeeds, two ounces;
Proof ſpirit, two gallons.
Let theſe ingredients ſteep together four days, and then draw off two gallons by diſtillation.

This is a very elegant aromatic water,

3

In the 21st Century …

Operations and concepts in medicinal chemistry are ‘industrialised’.

- Sufficient exposure at site of action.
- Proof of target engagement.
- Expression of functional pharmacological activity.

Bunnage et. al., *Nature Chem Biol*, **2013**, *9*, 195-199.
Morgan et. al., *Drug Discovery Today*, **2012**, *17*, 419-424.

Academic laboratories can contribute with more speculative ventures.
Three examples of projects at Strathclyde in areas of translational potential
– *modulation of biological function based upon natural products.*

- Nitric oxide synthase activators – defined molecular target.
- The ‘Worms’ project – possible molecular target.
- Minor groove binders for DNA – molecular target with multiple sites.

We don’t always know exactly how drugs work

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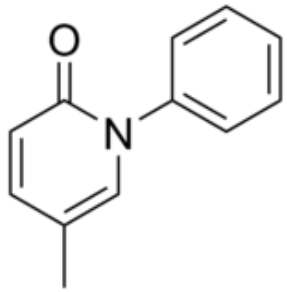
Pirfenidone

•Well-established antifibrotic and anti-inflammatory properties in various in vitro systems and animal models of fibrosis.

• Cell-based studies have shown that pirfenidone reduces fibroblast proliferation, inhibits TGF-β stimulated collagen production and reduces the production of fibrogrenic mediators such as TGF-β.

• Shown to reduce the production of inflammatory mediators such as TNF-α and IL-1β in both cultured cells and isolated human peripheral blood mononuclear cells.

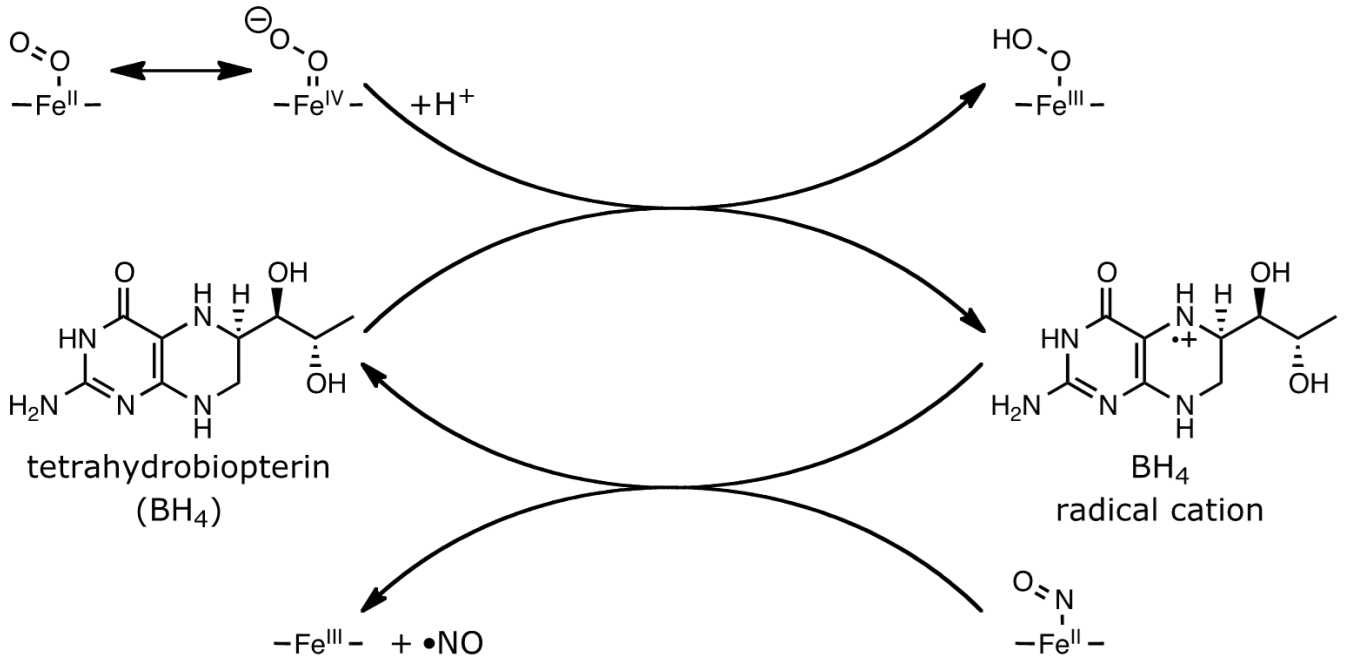
• Activities are consistent with the broader antifibrotic and anti-inflammatory activities observed in animal models of fibrosis.



Nitric oxide synthase activators

Three isoforms of NOS (endothelial NOS (eNOS), inducible NOS (iNOS) and neuronal NOS (nNOS).

All isoforms of NOS require the cofactors: **tetrahydrobiopterin (BH_4) & haem** also NADPH, FMN, FAD, CaM.



Potential clinical relevance of NOS activators

- Without BH_4, NOS cannot form the active dimer.
- Under BH_4 deficient conditions, superoxide ($O_2^{(\bullet-)}$) is generated not NO.
- $O_2^{(\bullet-)}$ depletes BH_4 levels (BH_4 is labile & readily oxidised).
- BH_4 depletion is implicated in some diseases (atherosclerosis, diabetes, and hyperlipidaemia).

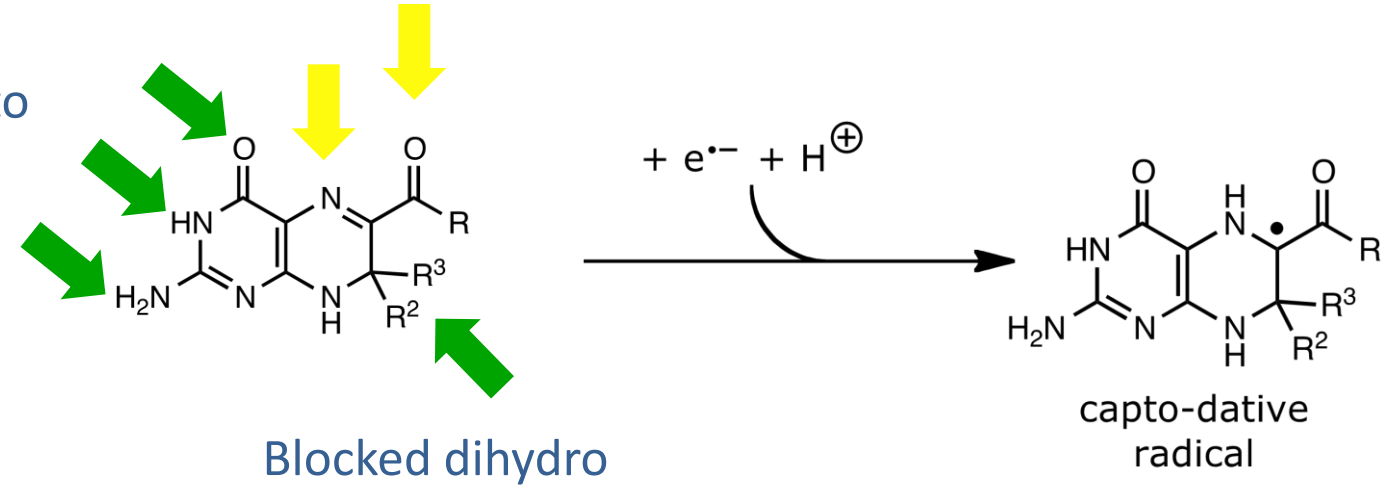
- Some drugs have been developed to enhance the therapeutic potential of NO. These lack selectivity.
- Administering BH_4 (unstable) or its precursor sepiapterin in patients with endothelial dysfunction restores function and prevents atherosclerotic lesions.

- Still an unmet need to treat atherosclerosis, even under optimal therapy (statins, aspirin, blood pressure control antidiabetic drugs) still 30-50% vulnerable to vascular event.

Design rationale

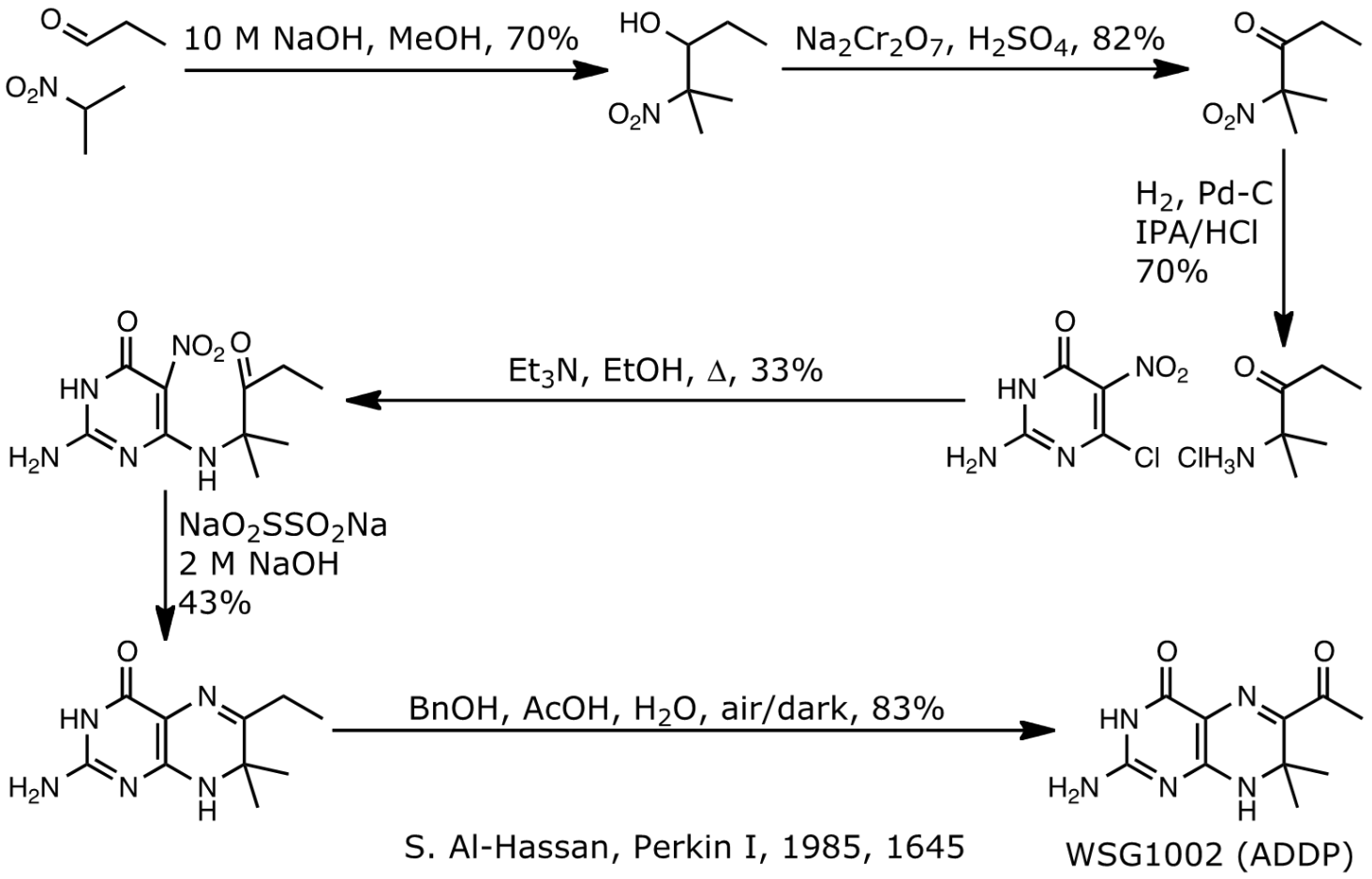
Stabilized radical?

Important for binding to NOS



Blocked dihydro oxidatively stable

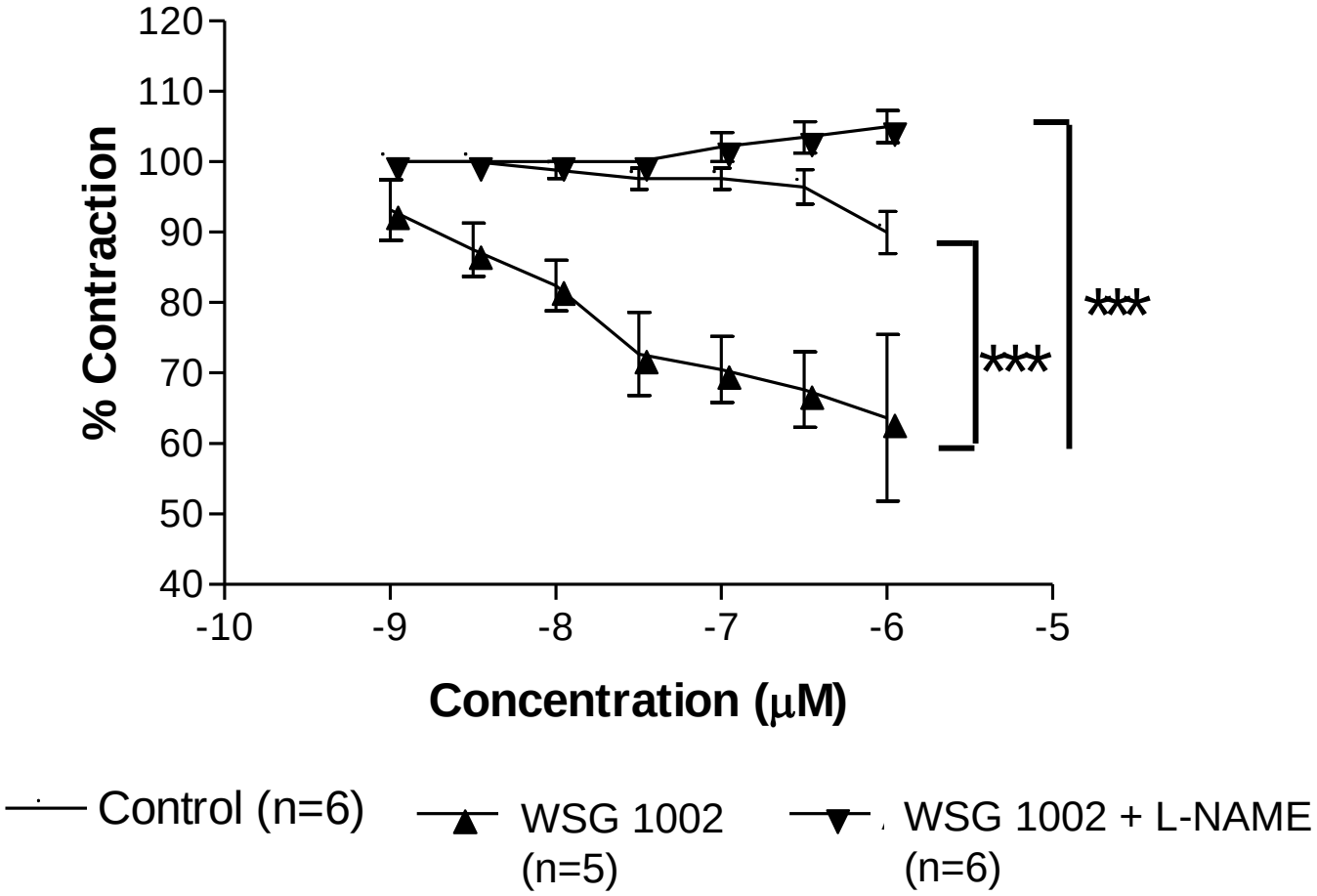
Synthesis of oxidatively stable dihydropterins



S. Al-Hassan, Perkin I, 1985, 1645

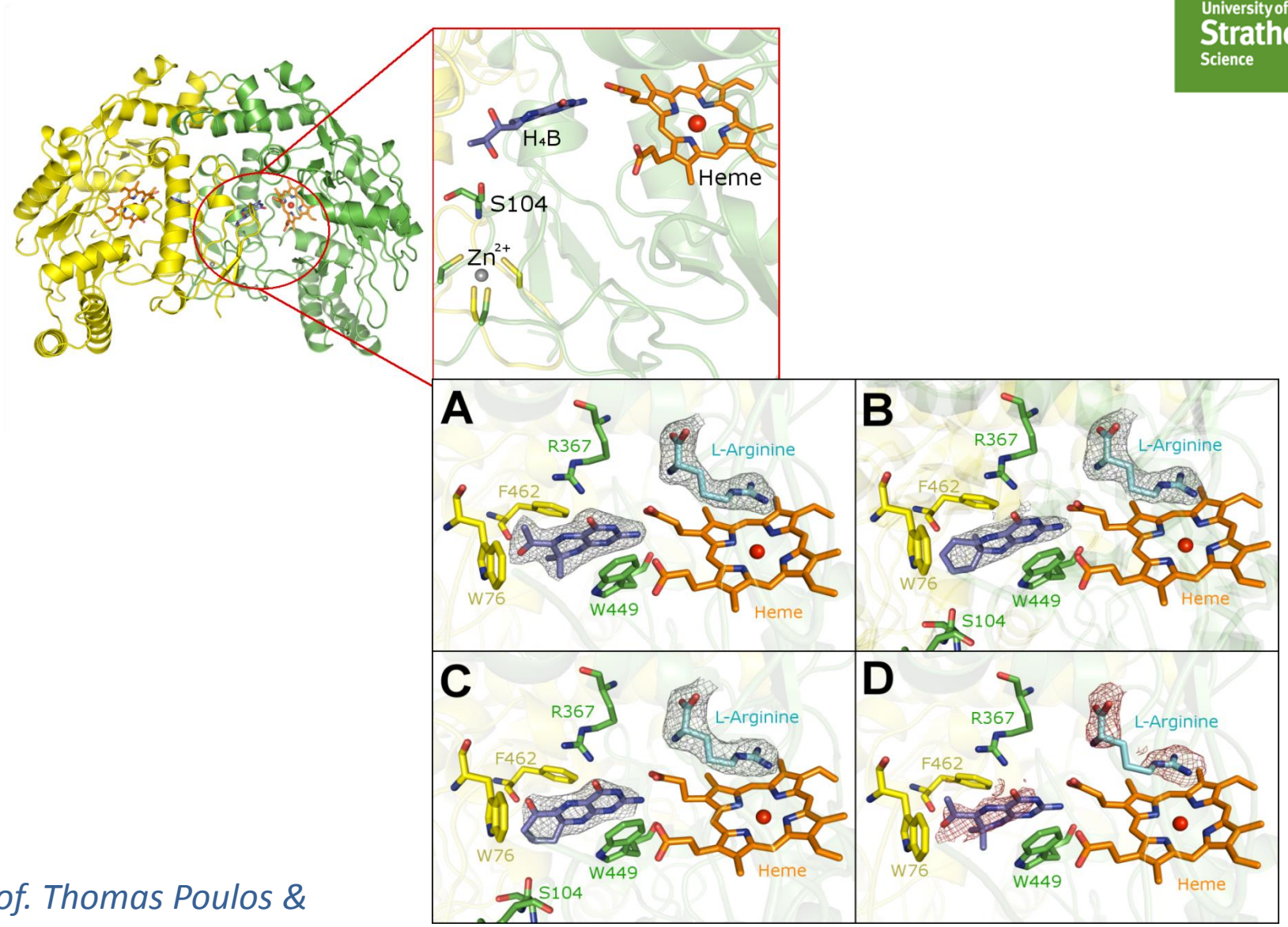
WSG1002 (ADDP)

WSG1002 (= ADDP) relaxes rat aorta



Prof. Roger Wadsworth & Dr Suma Kunuthur

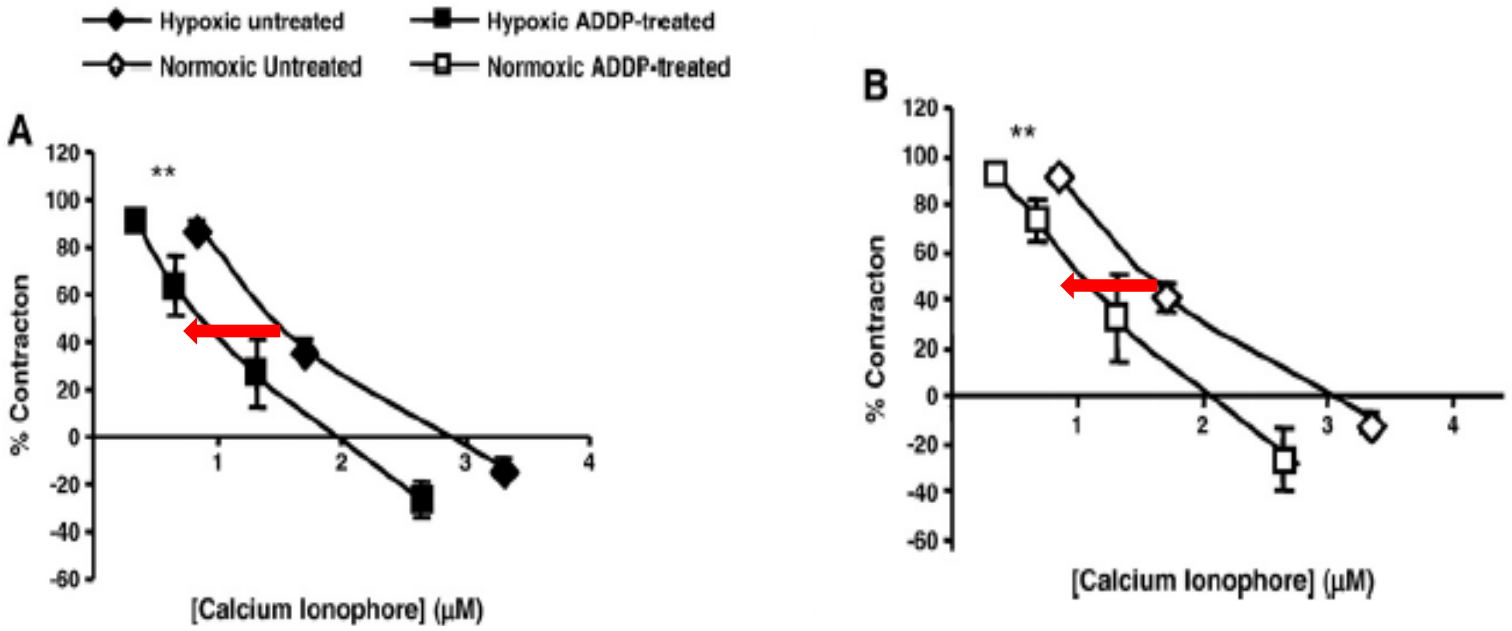
BH_4 analogues bind like BH_4 itself



Prof. Thomas Poulos &
Dr. Huiying Li, UC Irvine

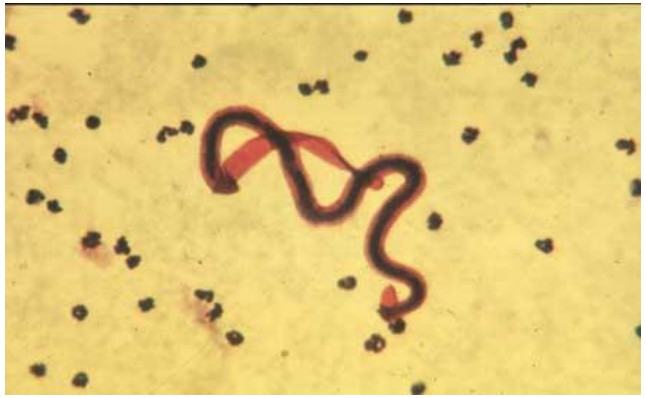
BH_4 analogues relax pulmonary arteries

Preconstricted arteries respond better to Ca ionophore A23187 after *in vivo* treatment with ADDP (wsg1002) in both normoxic and hypoxic rats.



Prof. Roger Wadsworth & Dr Suma Kunuthur

The ‘Worms’ Project



ES-62: An immunomodulatory protein secreted by the filarial nematode *Acanthocheilonema viteae*.

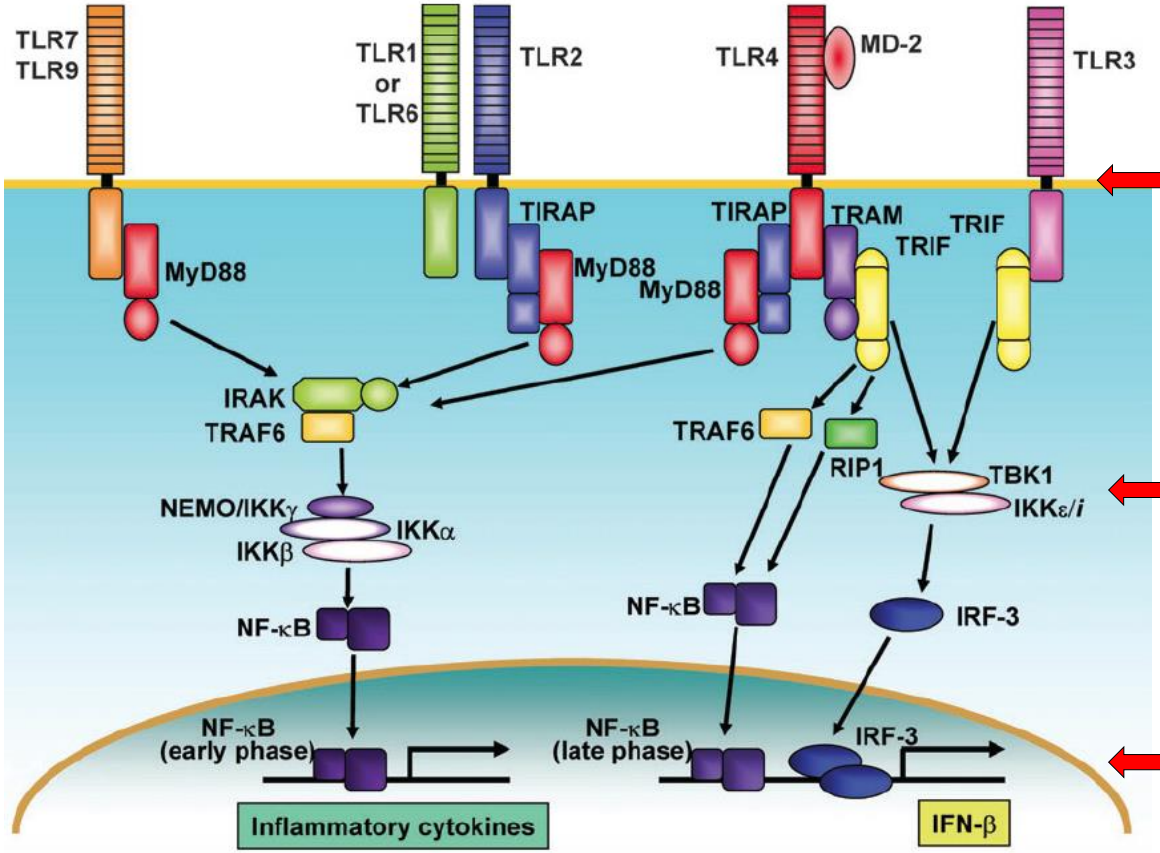
Activity is dependent on a post-translational modification of phosphorylcholine attachment to an *N*-type glycan.

Parasites, including worms and protozoa, modulate the immune response of the host by secreting proteins that often have ***phosphoryl choline***, or a closely related structure.

Phosphoryl choline itself is widespread and would not give selectivity. It is also zwitterionic and not suitable for crossing cell membranes unaided.

Profs Billy and Maggie Harnett

Relevant field of biology

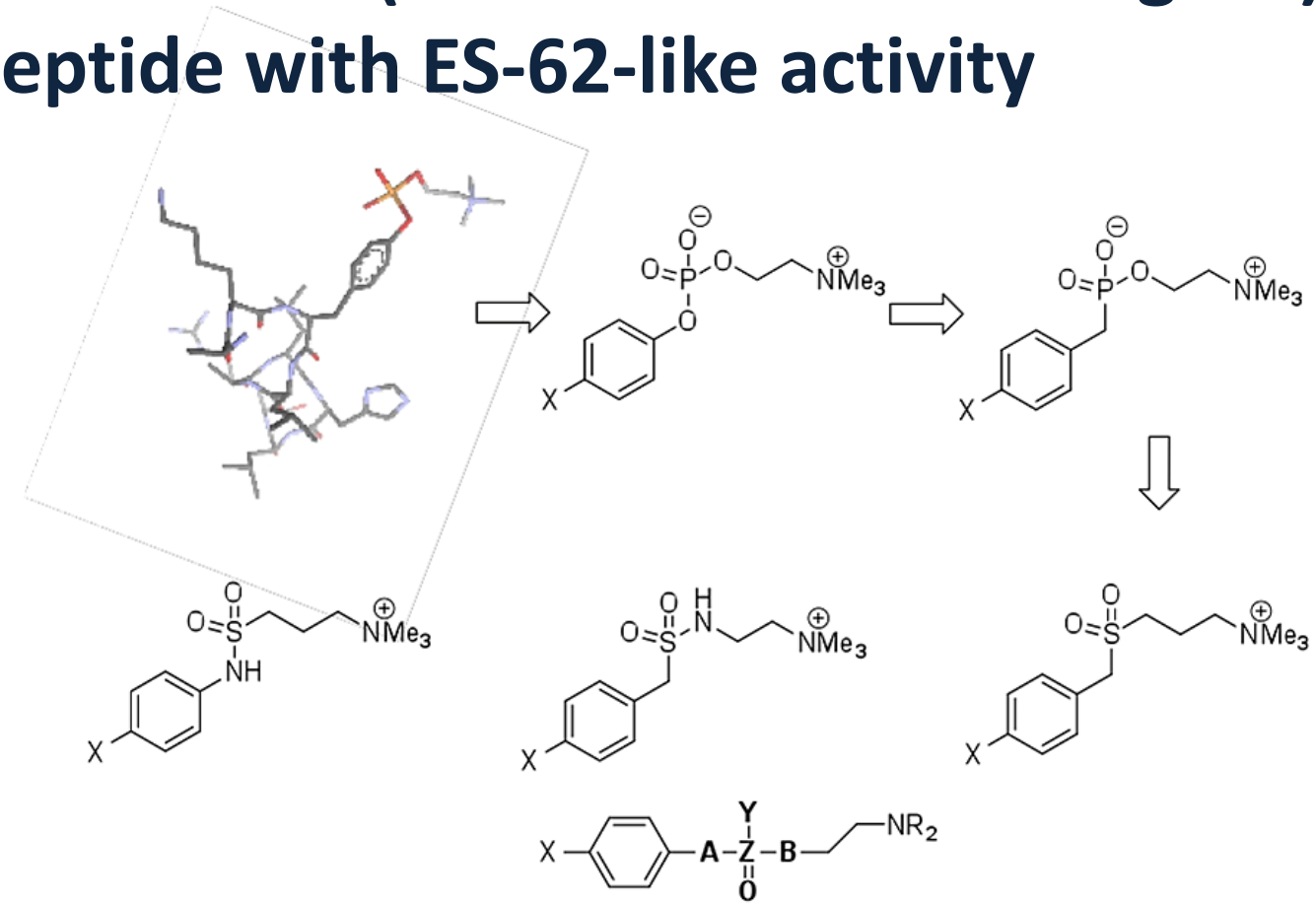


Toll-like receptors (TLR)
Adapter proteins (MyD88)

Kinase pathways

Cytokine release profile
(primary assay)

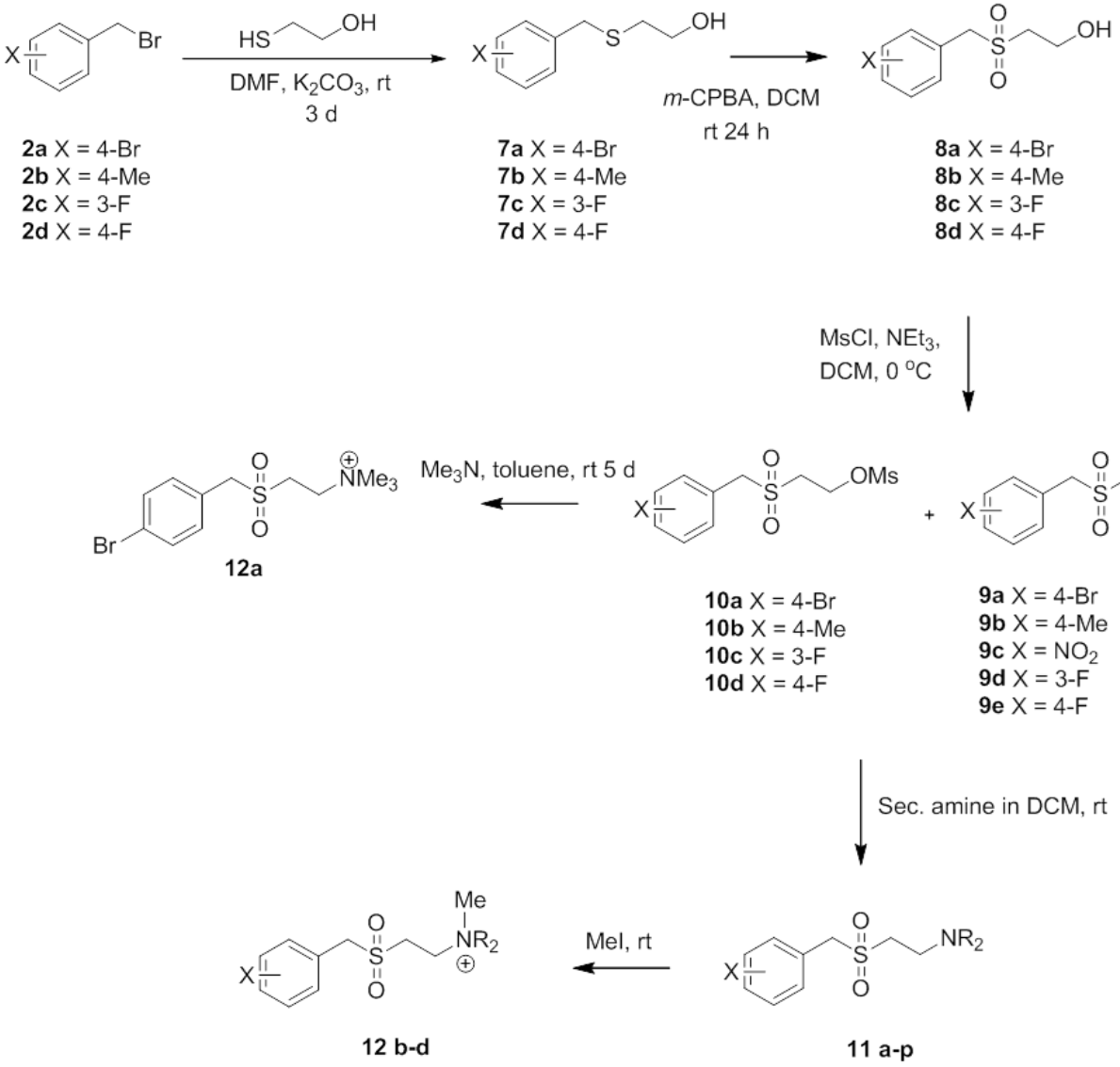
Origin of SMAs (small molecule analogues) – a peptide with ES-62-like activity



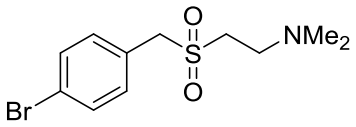
1

A = O, CH_2
B = nothing, CH_2
R = small alkyl, cycloalkyl
X = substituents as described in text
Y = OH, O
Z = P, S

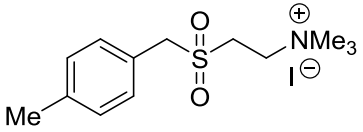
Typical synthesis



Proof of concept *in vivo* – asthma and arthritis

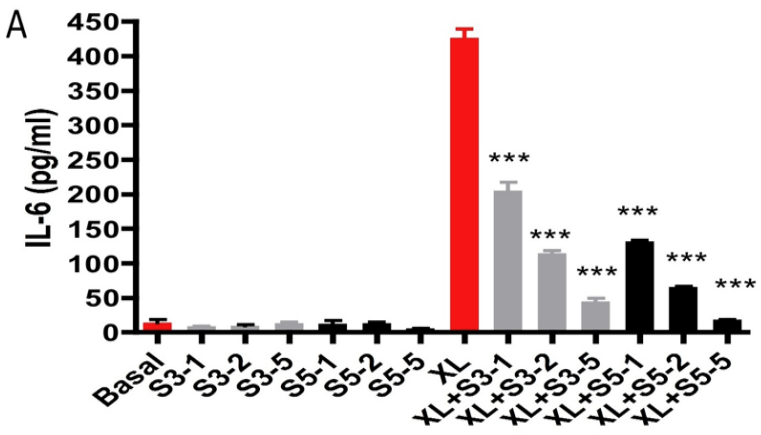


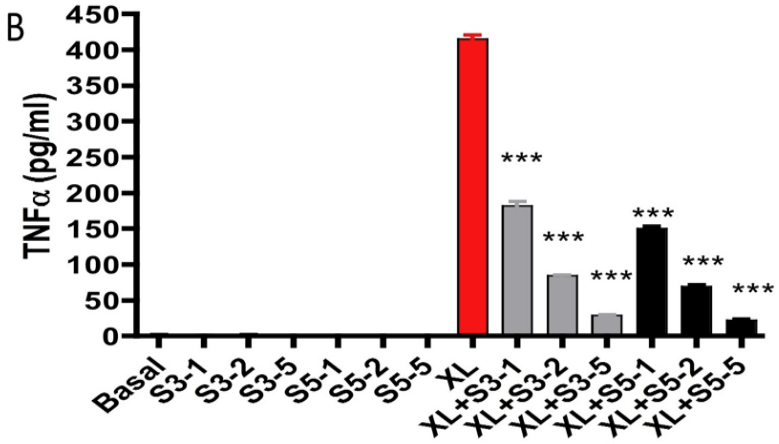
S3 or 11b

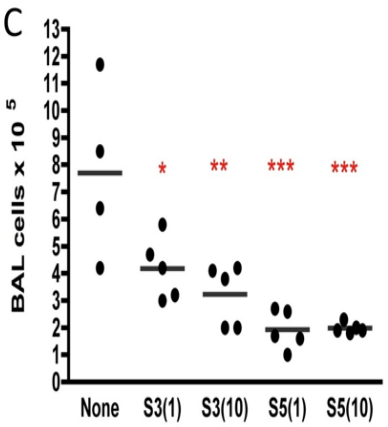


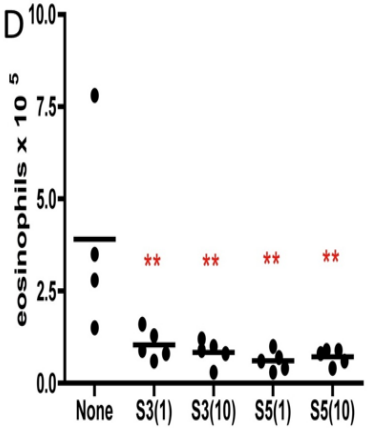
S5 or 12b

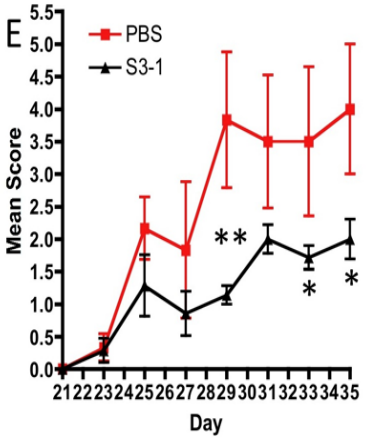
Cytokine release *in vitro*

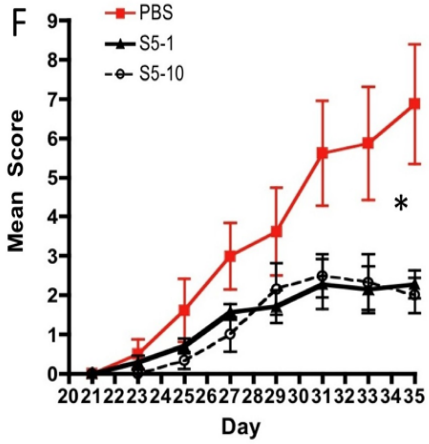






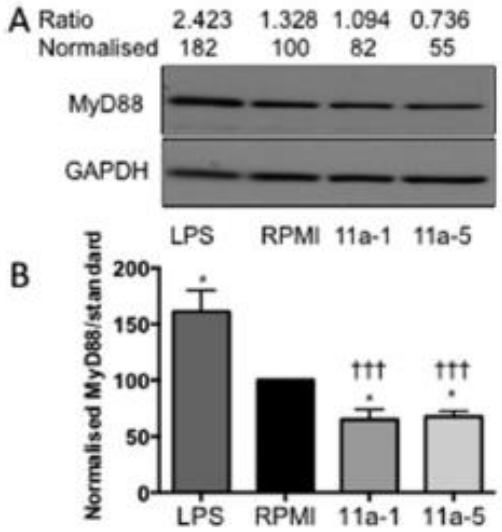


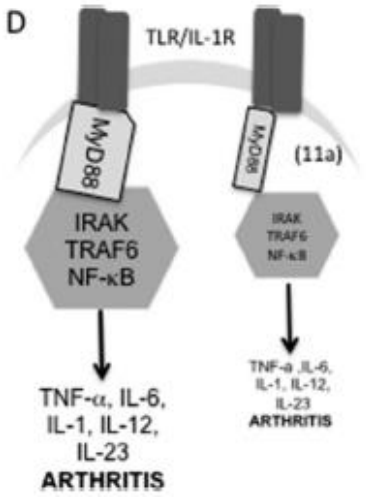




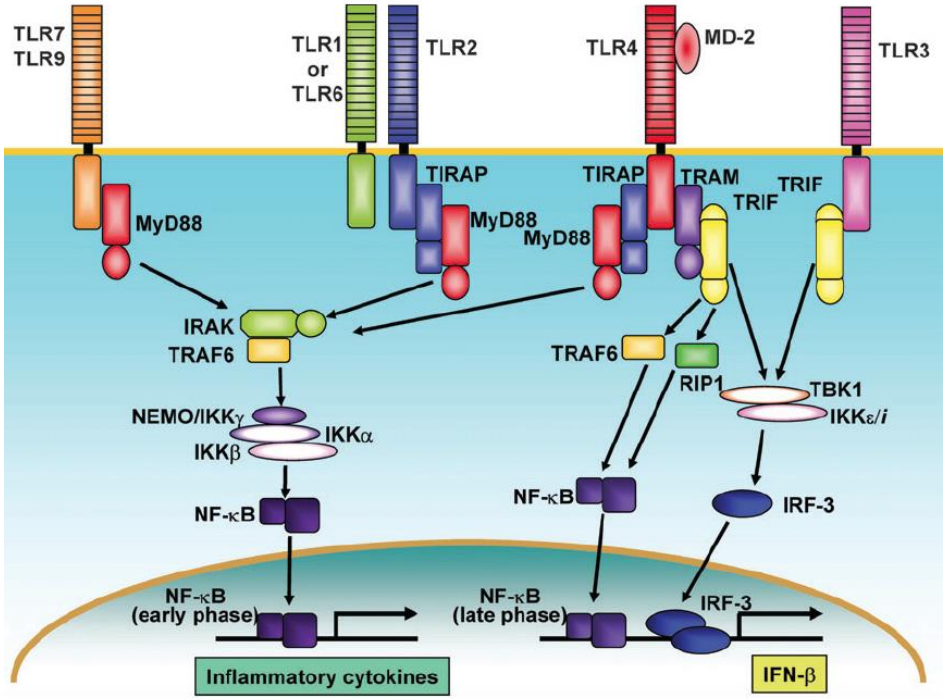
***In vivo* effects C and D = asthma, E and F = collagen induced arthritis**

Mechanism of action of ES-62 and SMAs

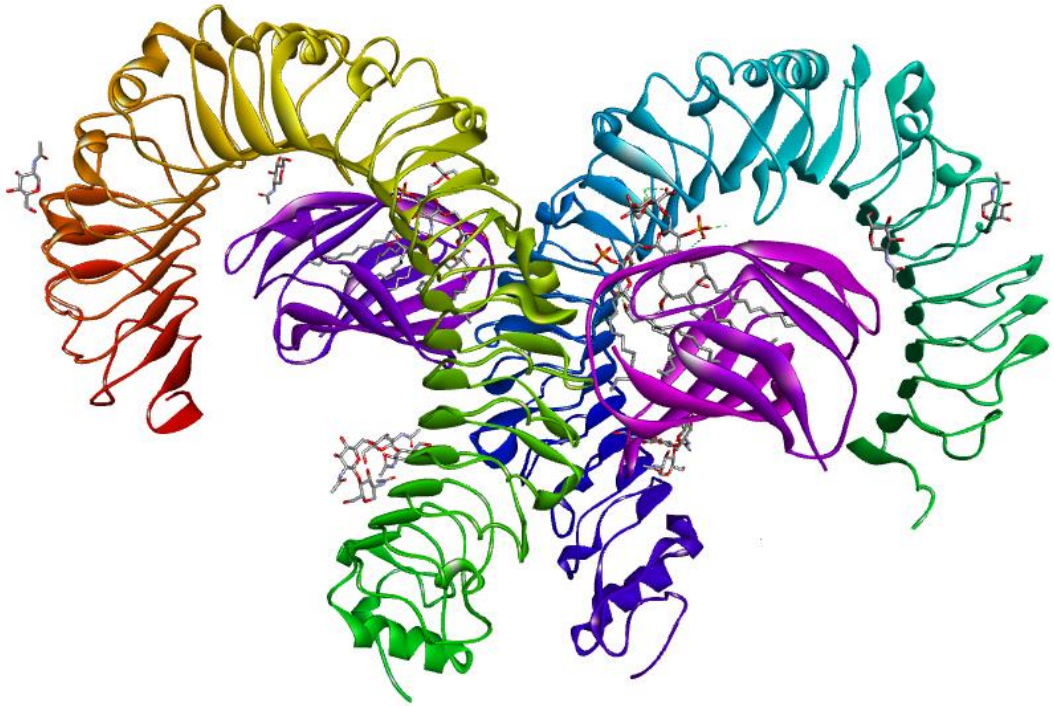




ES-62 and SMAs downregulate the MyD88 gene leading to a reduction in stimulation of the TLR/IL-1R pathway and consequent reduction in the release of pro-inflammatory cytokines.



TLR4 – a possible molecular target



Crystal structure of TLR4 dimer complexed with MD2 and LPS.
Indirect evidence that ES-62 binds to TLR4.
ES-62 and S3 do not modulate gene expression.
S5 modulates expression of some genes associated with inflammation.

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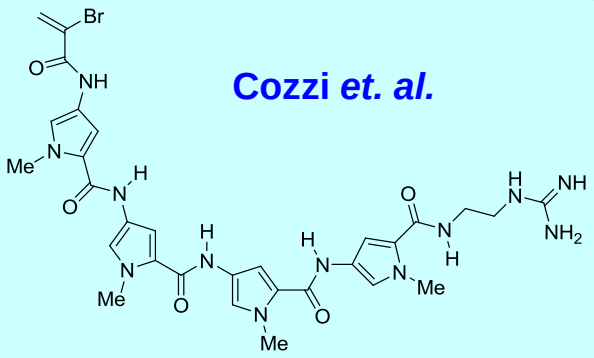
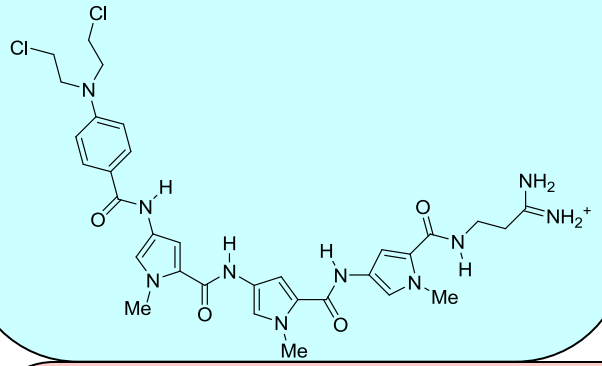
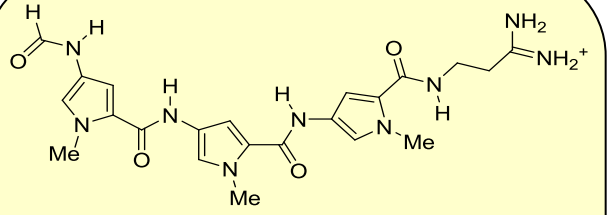
Polyamide MGBs

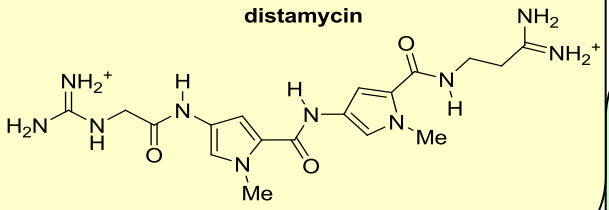


Cozzi *et. al.*

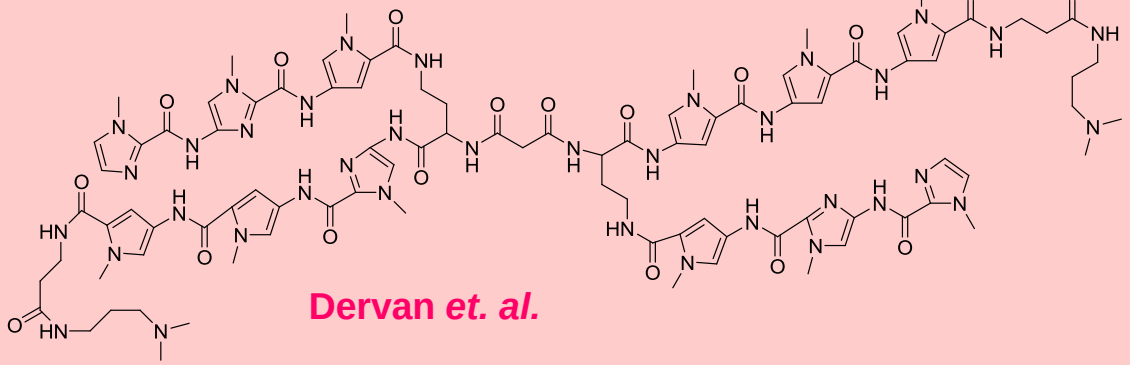




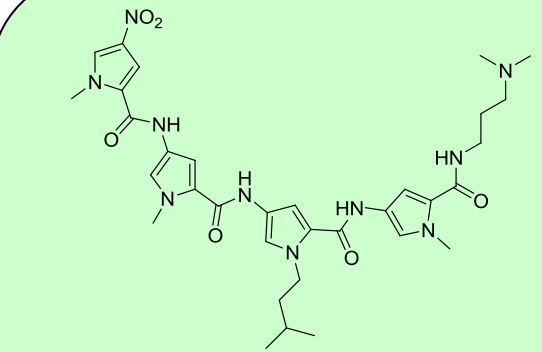
distamycin

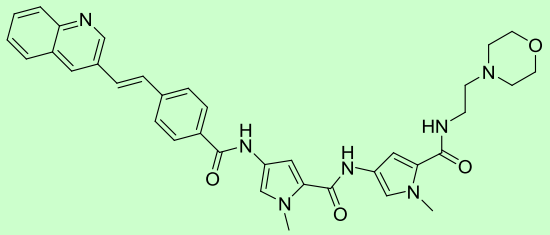


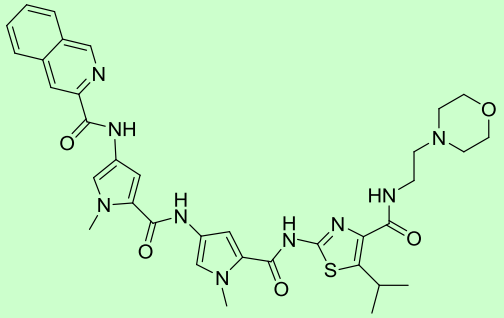
netropsin



Dervan *et. al.*

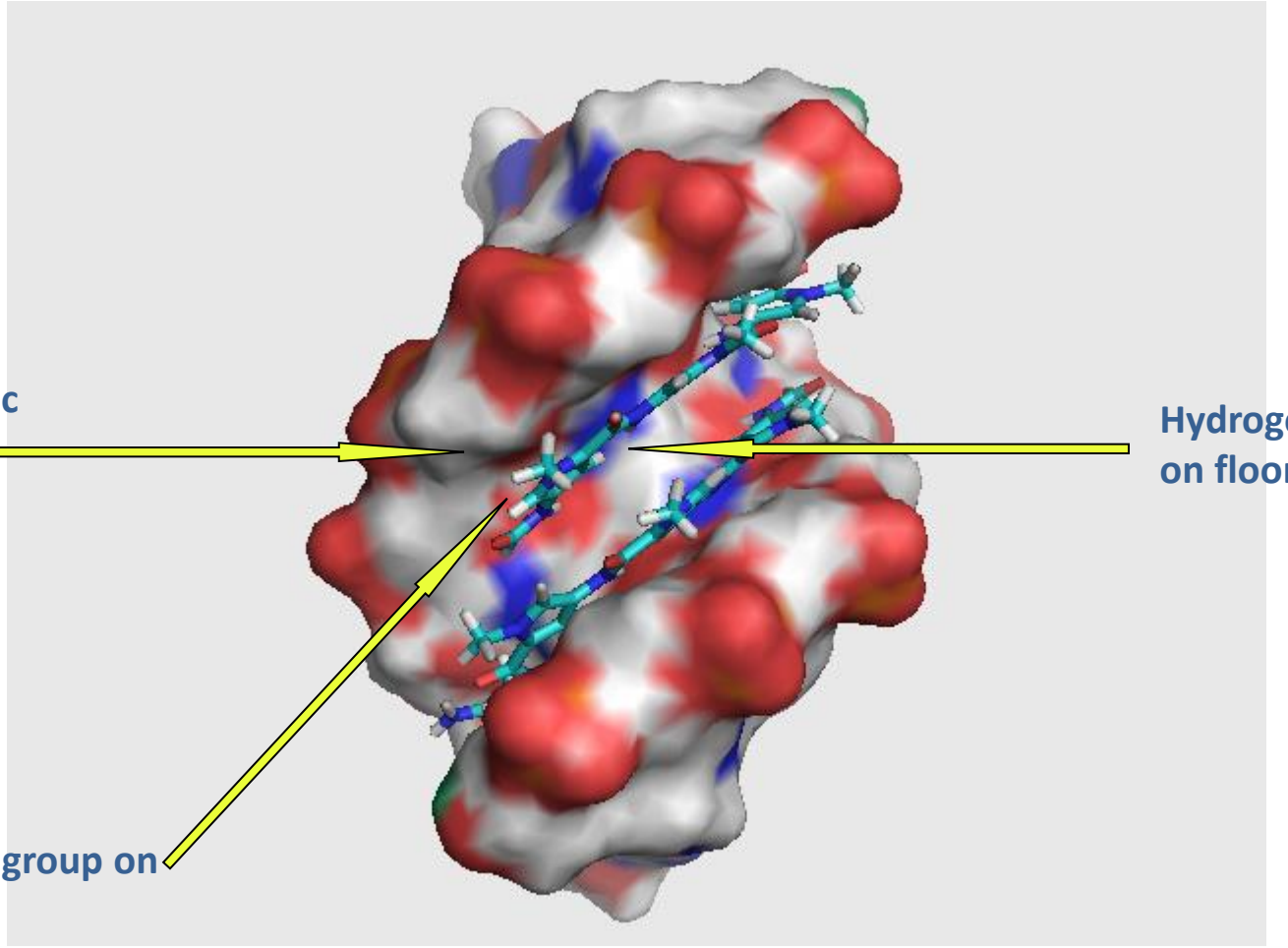






Strathclyde

Primary design concept at Strathclyde



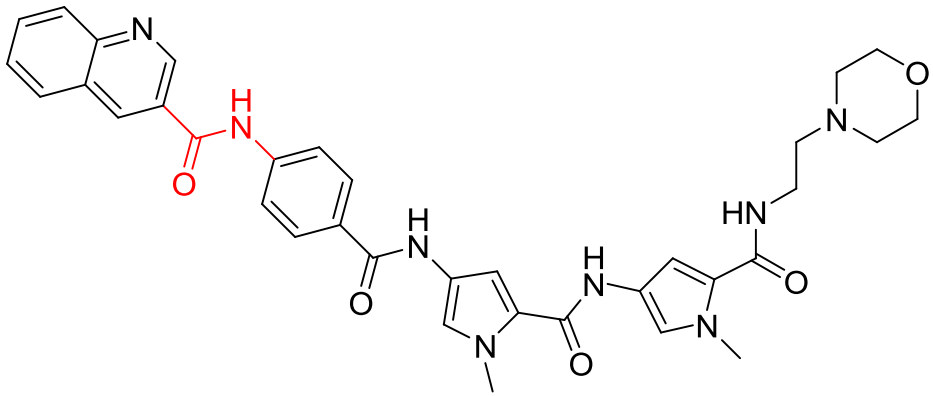
An amide isostere – the key structural change

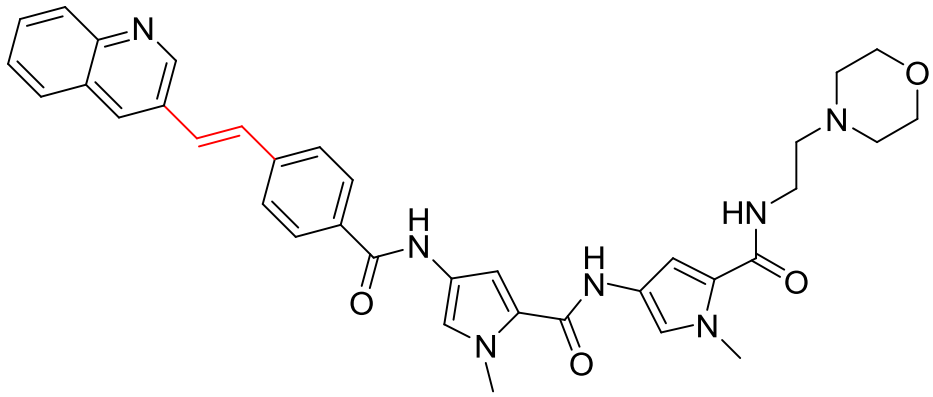
Amide: planar, H-bond donor and acceptor, hydrolysable.

Alkene: planar, non-polar, stable to hydrolysis.

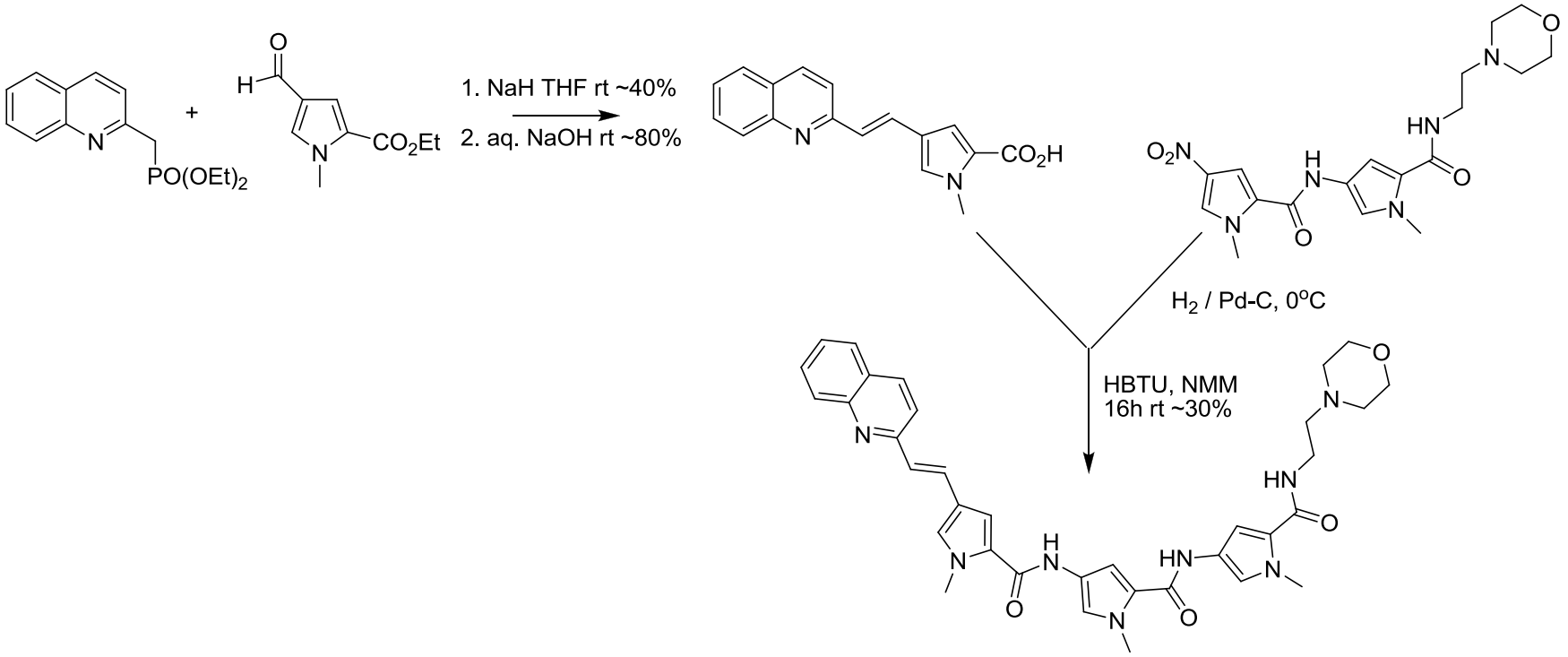
One hydrogen bond lost.

T_m measurements show that loss of a hydrogen bond does not weaken binding to DNA oligos in this group of compounds.

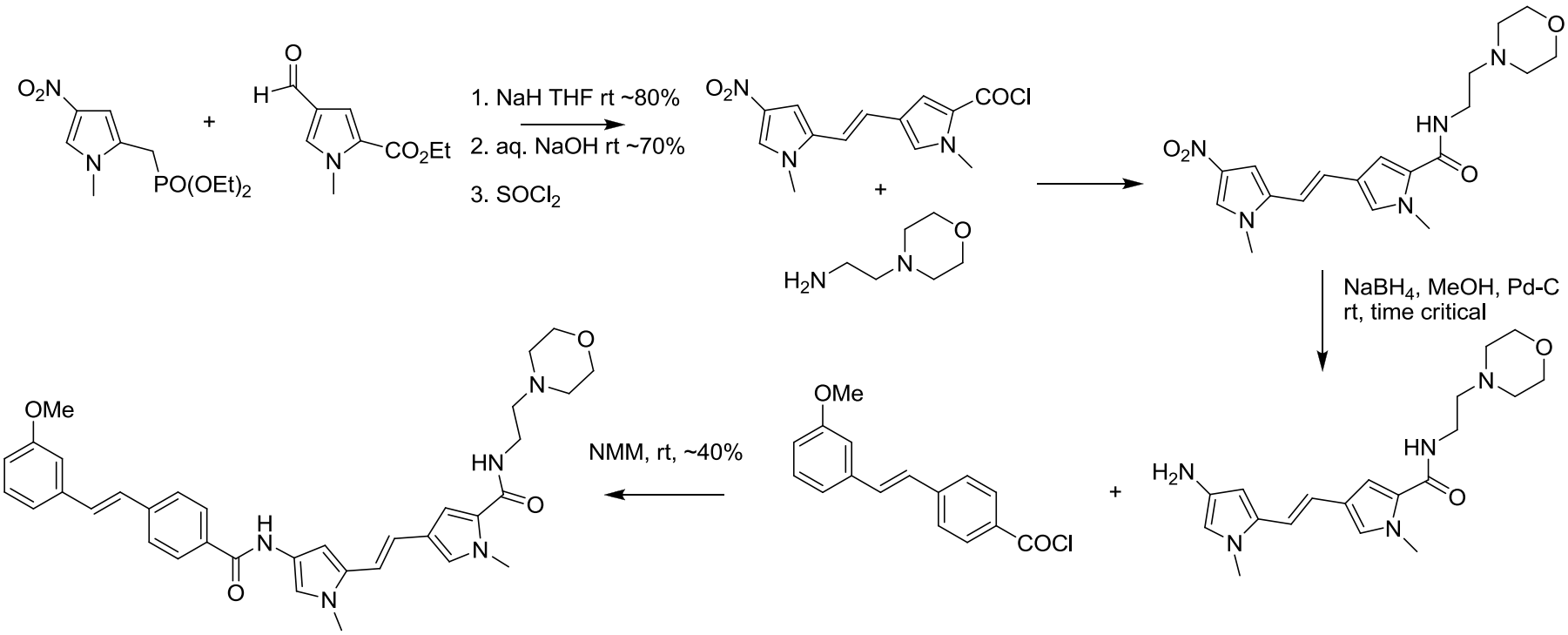




Typical synthesis of head group alkene

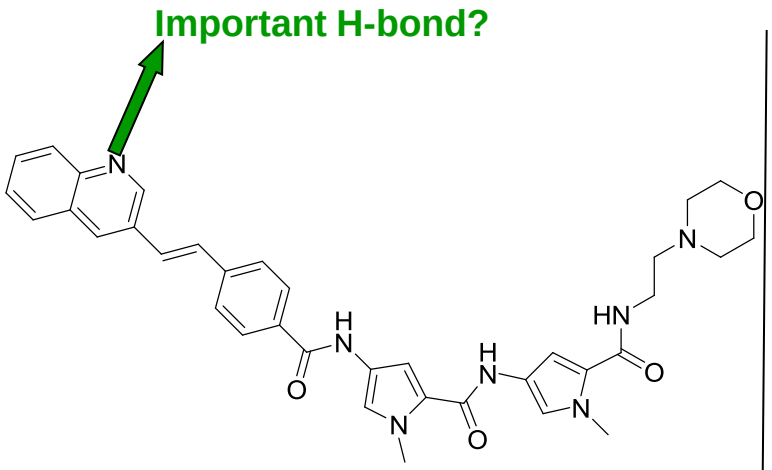


Typical synthesis of ‘internal’ alkene



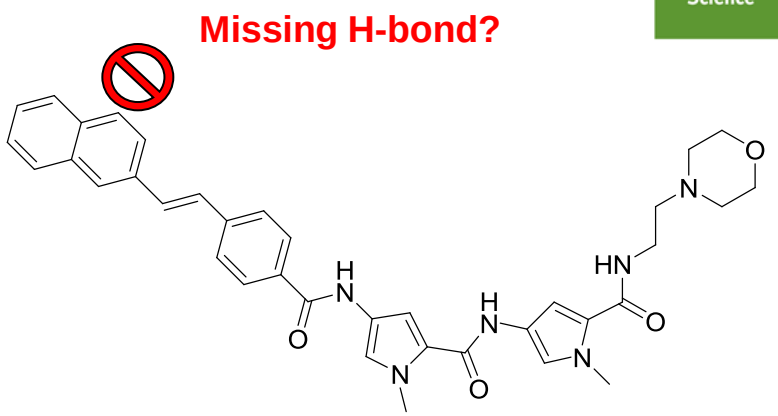
Dr Abed Khalaf, Dr David Breen

Some possible key points of interaction



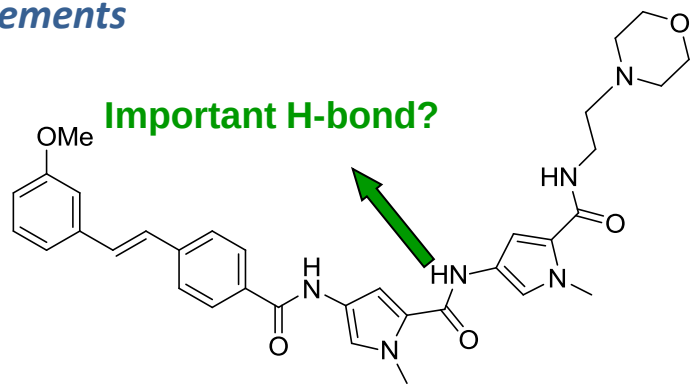
Active antibacterial compounds.

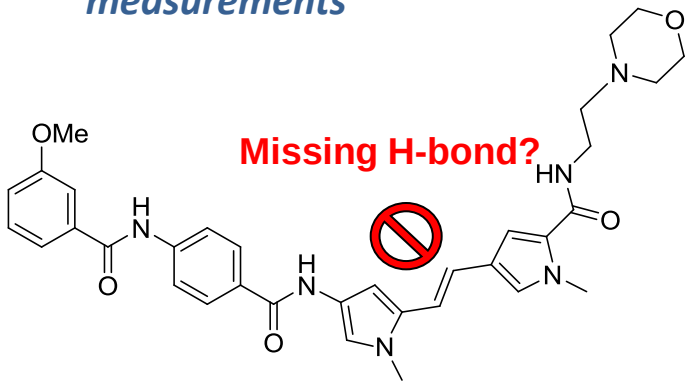
Bind to target DNA as shown by T_m measurements



Inactive antibacterial compounds.

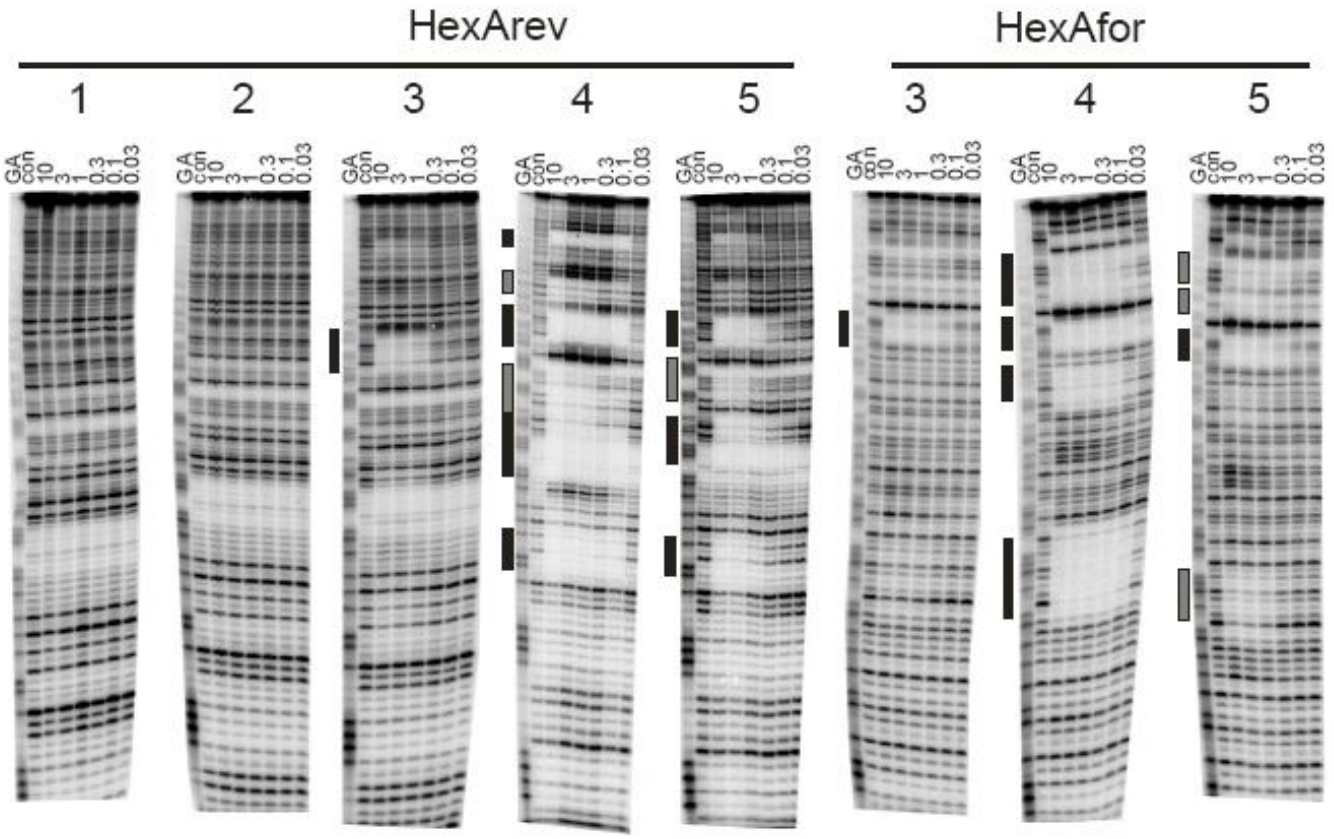
Do not bind to target DNA as shown by T_m measurements

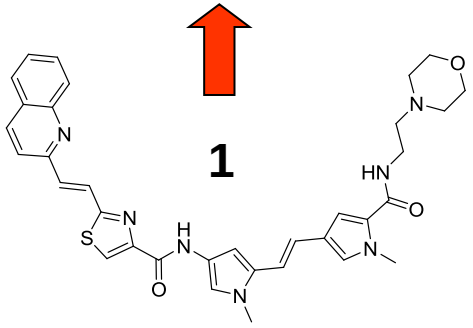


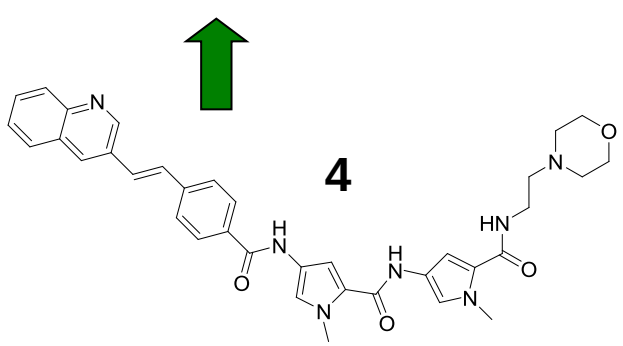


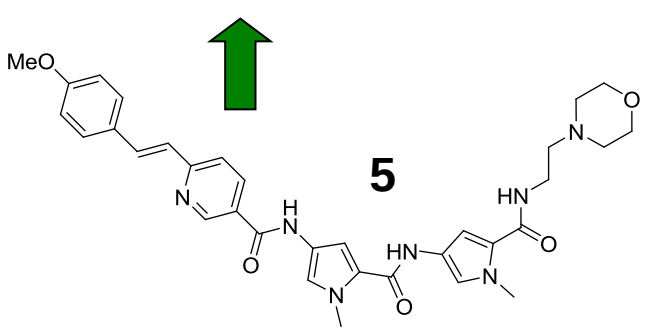
Target oligonucleotide = GCGATATATGCG/CGCTATATACGC

Footprinting evidence



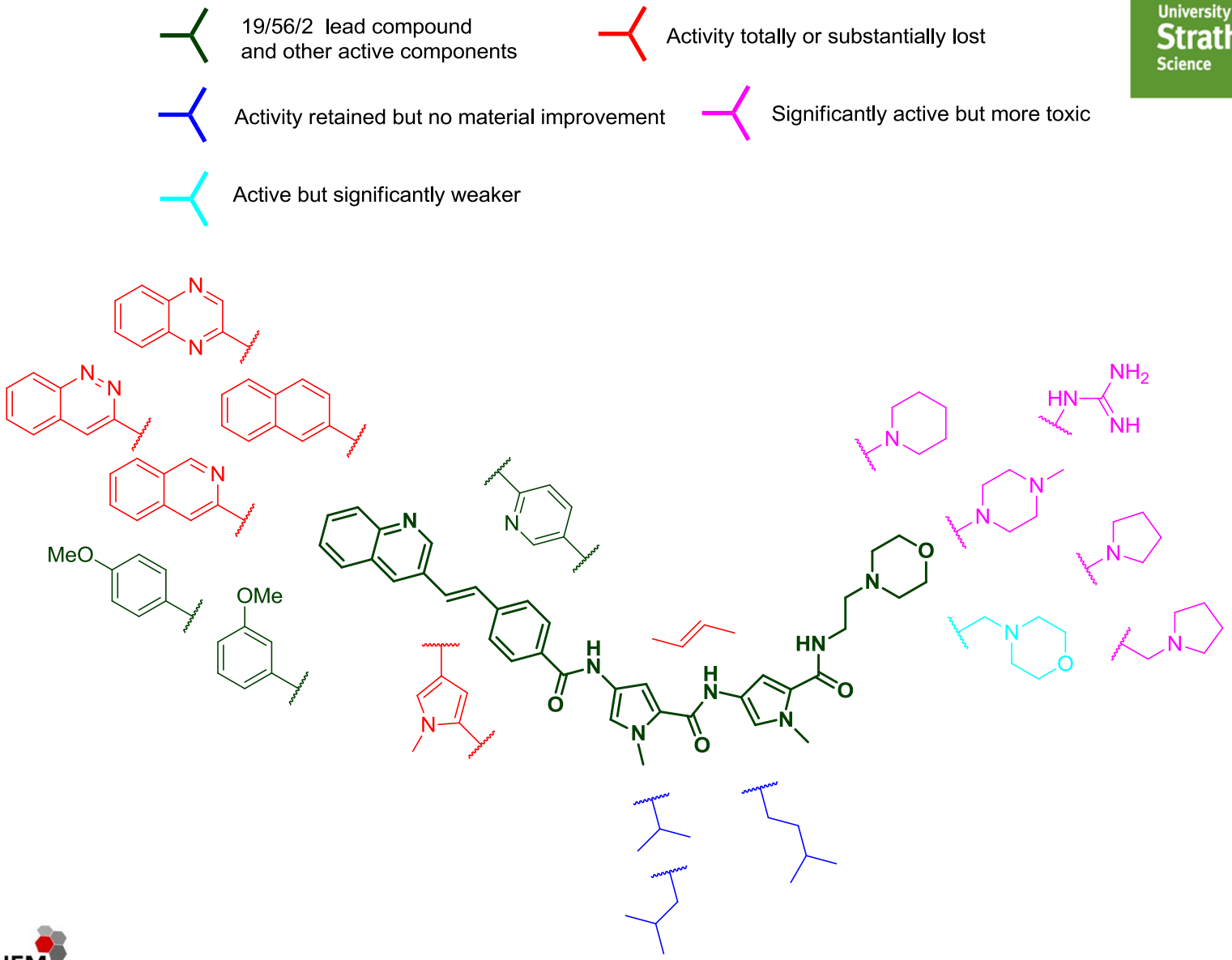






Prof Keith Fox, Southampton

SAR summary for antibacterial activity



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Species and resistant strains

Organism	MGB-BP-3				
	n=	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MBC_{50} (mg/L)	MBC_{90} (mg/L)
Group B Streptococci	15	0.25	1	0.25	1
Group C Streptococci	15	0.25	1	0.5	1
Group G Streptococci	15	0.5	0.5	0.5	0.5
Methicillin-resistant *Staphylococcus aureus*	15	1	2	1	2
Methicillin-resistant *Staphylococcus epidermidis*	15	0.25	0.5	0.5	2
Methicillin-susceptible *Staphylococcus aureus*	15	0.5	1	1	2
Methicillin-susceptible *Staphylococcus epidermidis*	15	0.25	0.5	0.25	2
Streptococcus constellatus	15	0.25	0.5	0.5	1
Streptococcus mitis	15	0.5	2	0.5	2
Streptococcus pyogenes	15	0.25	0.5	0.25	2
Vancomycin-resistant *Enterococcus faecalis*	15	2	2	>32	>32
Vancomycin-resistant *Enterococcus faecium*	15	1	2	>32	>32
Vancomycin-susceptible *Enterococcus faecalis*	15	1	2	>32	>32
Vancomycin-susceptible *Enterococcus faecium*	15	1	2	>32	>32

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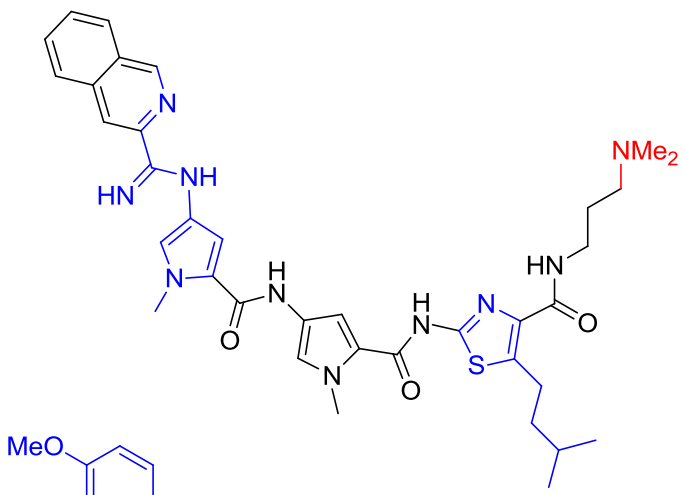
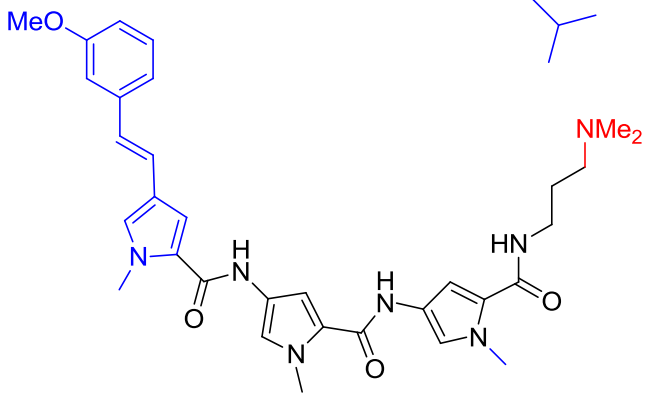
Antifungal activity



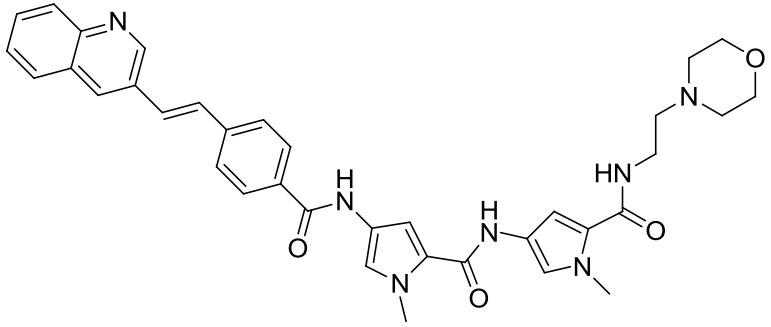
MIC = 6.25 μM vs. *Candida, Aspergillus*

MIC > 50 μM for bacteria



MIC = 1.6 μM vs. *Candida*

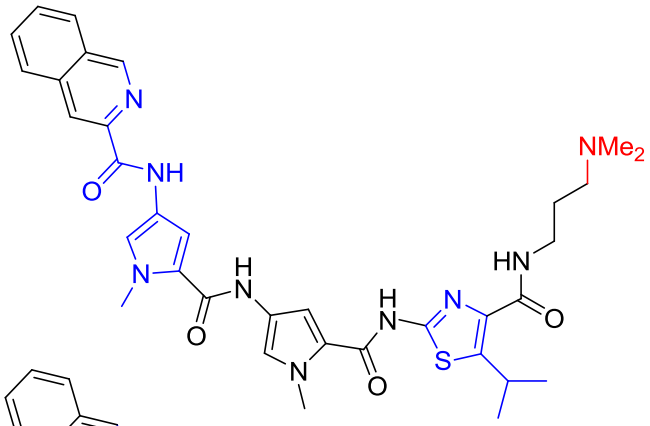
MIC ~ 20 μM for bacteria



Lead antibacterial compound

MIC > 50 μM for fungi

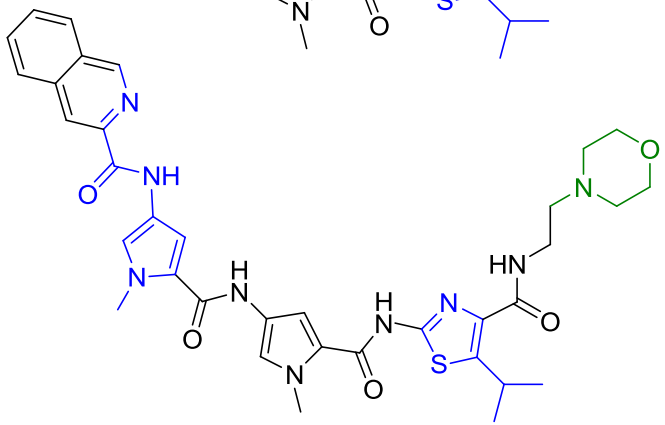
More unexpected selectivity - *Trypanosoma*



MIC = 3.1 μM vs. *Trypanosoma brucei*

Toxic to mammalian cells at 20 μM

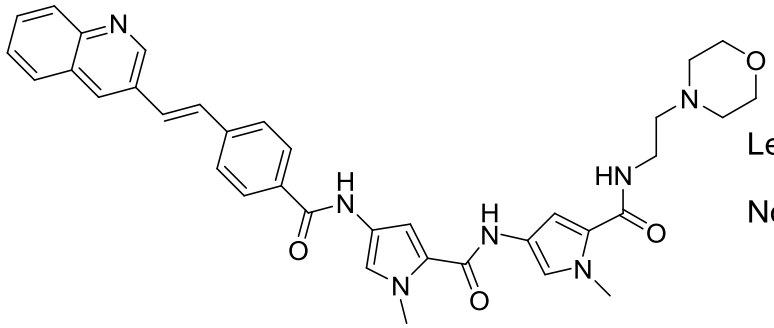
MIC > 50 μM for bacteria and fungi



MIC = 0.4 μM vs. *Trypanosoma brucei*

No toxicity to mammalian cells at 20 μM

Inactive against bacteria and fungi



Lead antibacterial compound

Not significantly active against Tryps

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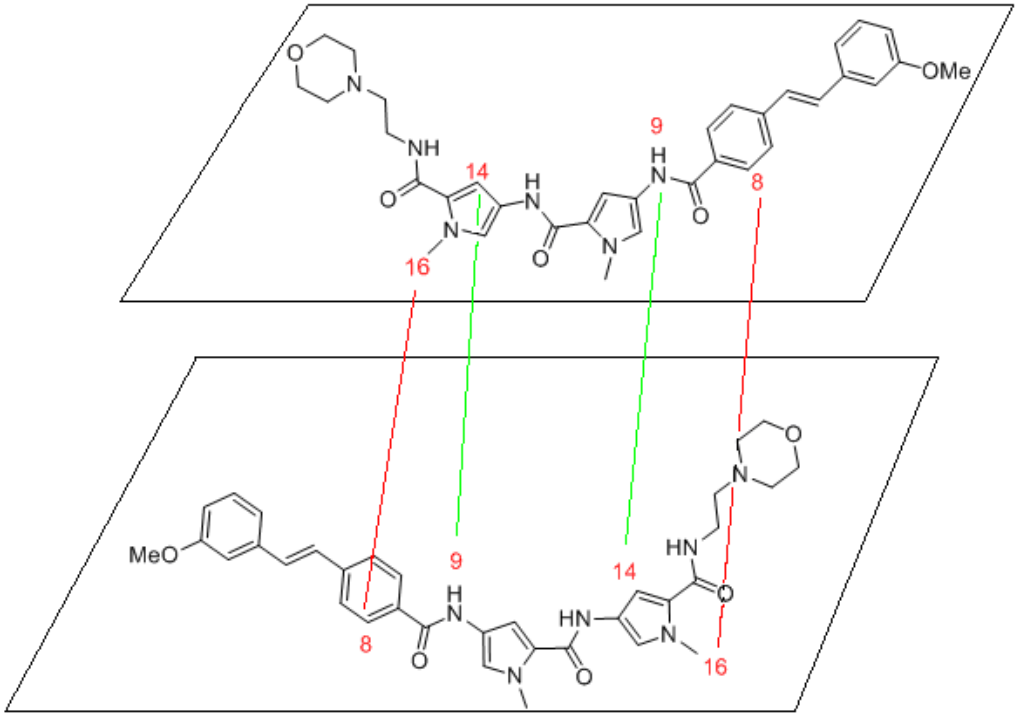
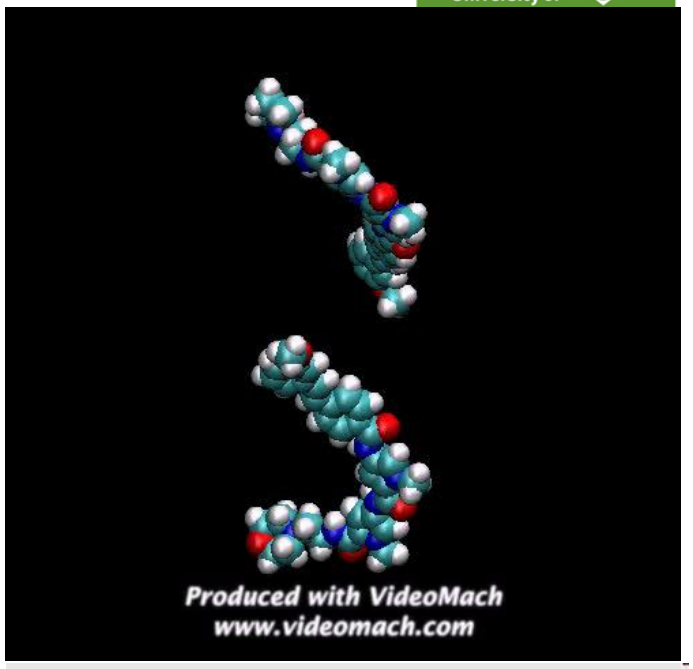
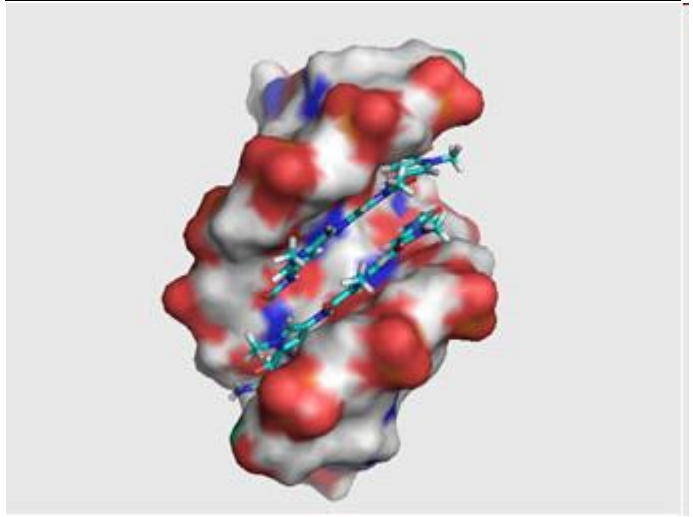
Aggregation and formulation





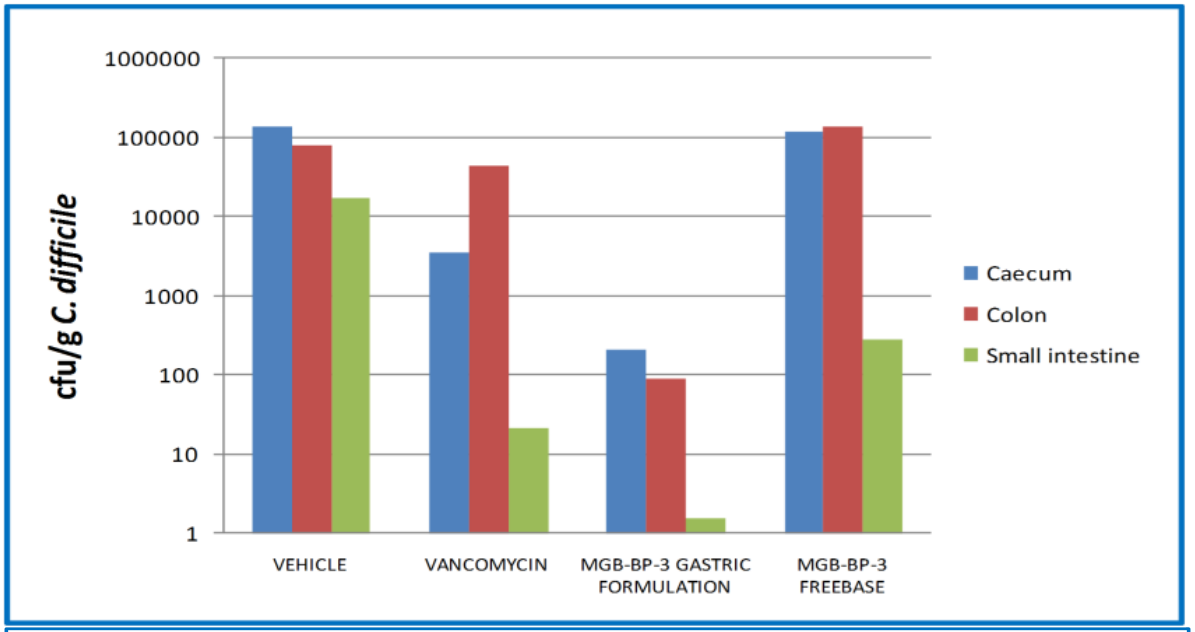


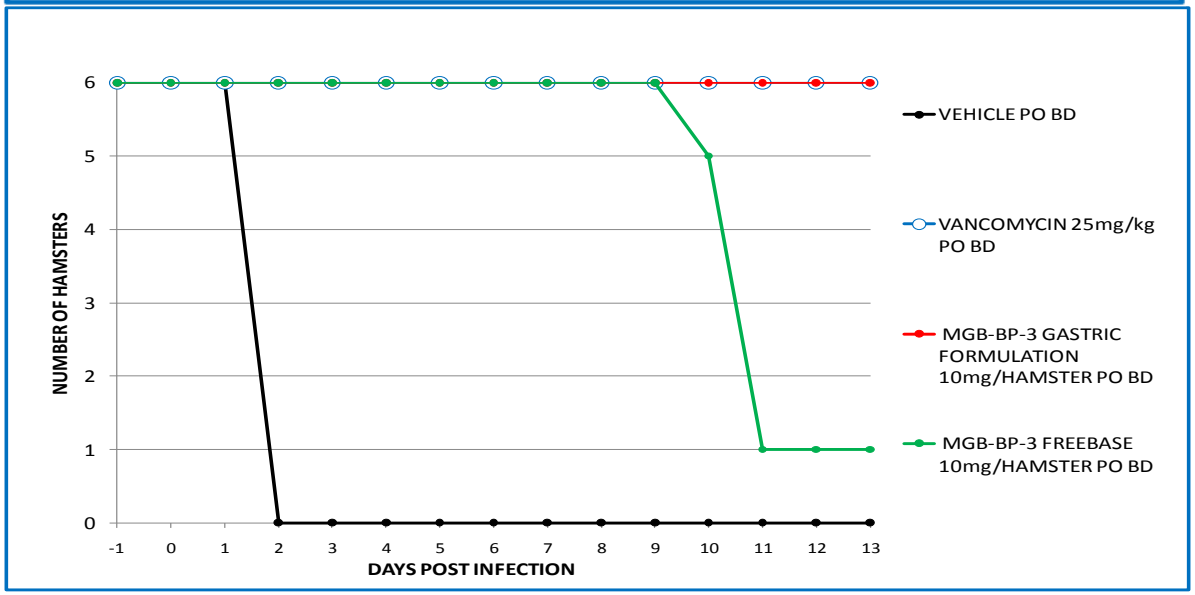
NMR results consistent with MGBs' entering binding site as a dimer, not sequentially.

Gail Wilson, Dr John Parkinson

WestCHEM

Formulated drug against *C. difficile*





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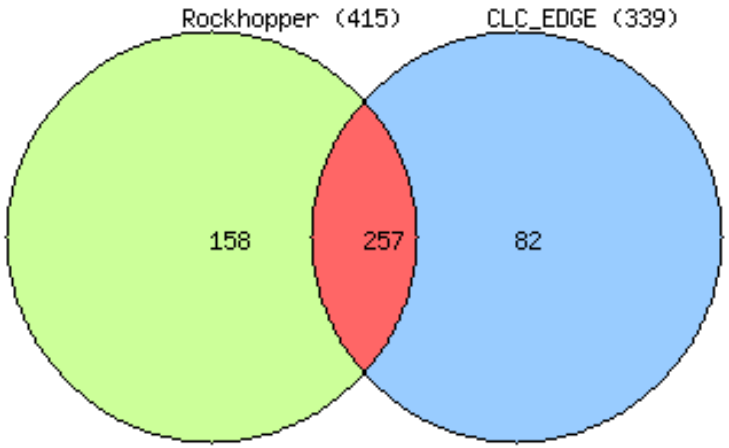
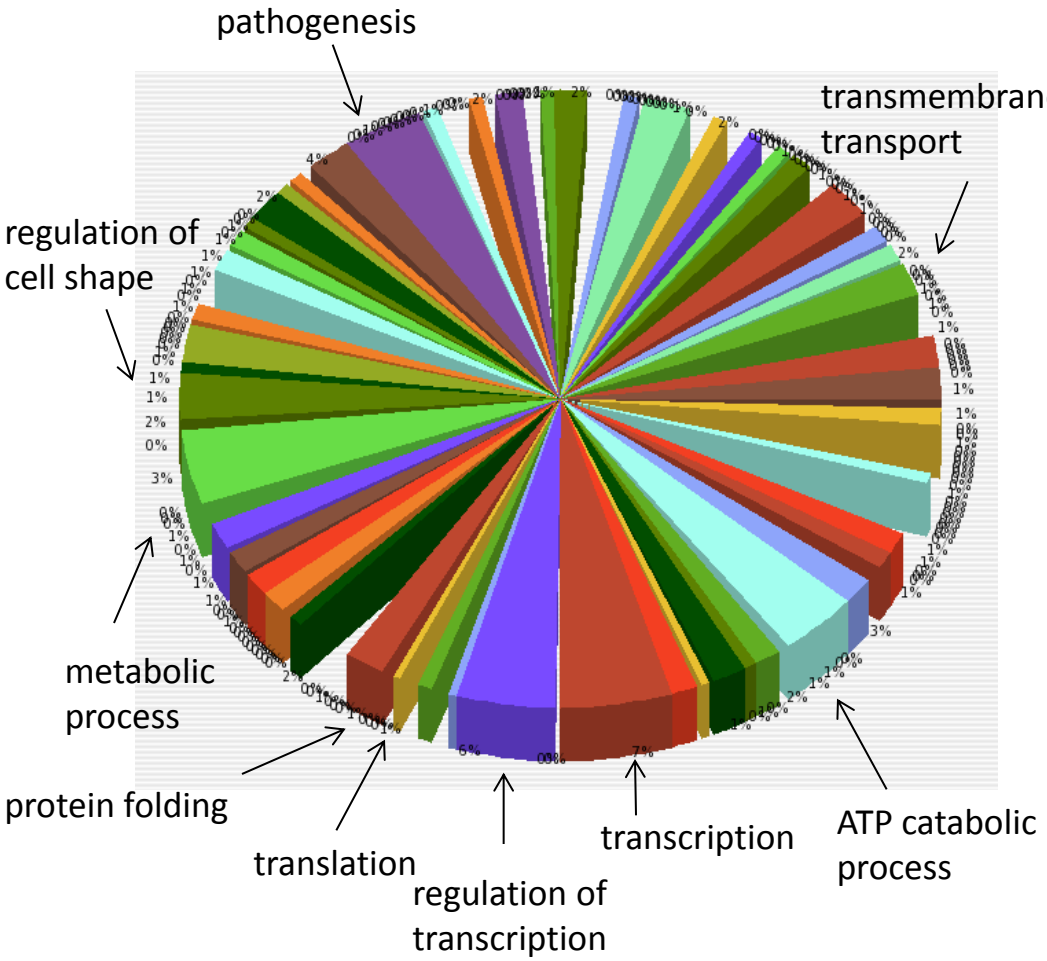
Genes significantly downregulated

- 257 downregulated genes common to both data analysis methods





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