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Selectivity in Anti-infective Minor Groove Binders

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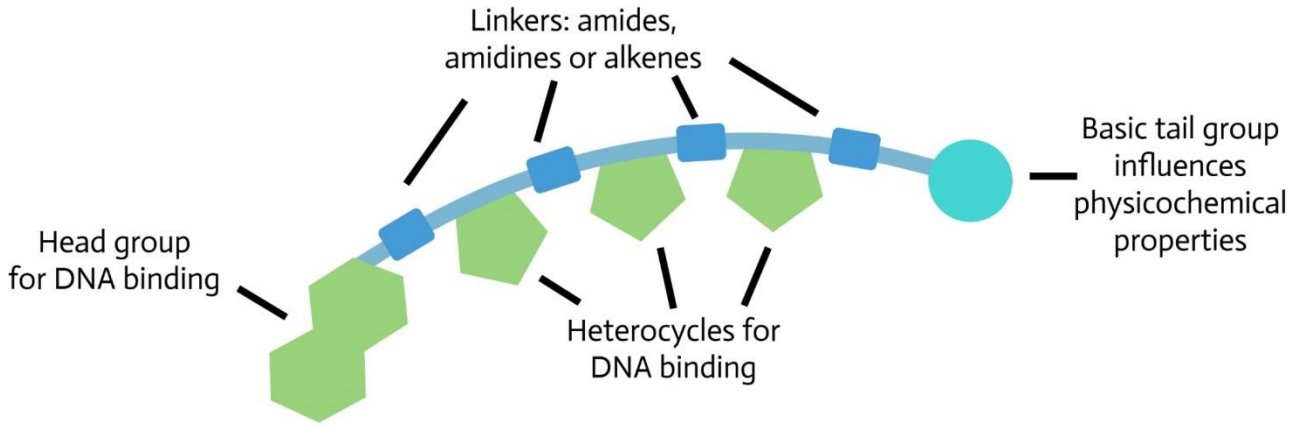
Selectivity in Anti-infective Minor Groove Binders

Graphical Abstract

The Minor Groove Binder



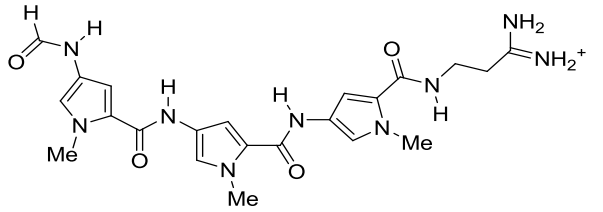
Abstract: Minor groove binders for DNA synthesised at the University of Strathclyde (S-MGBs) have been successfully shown to be active against a wide range of infectious organisms including bacteria, fungi, and parasites in particular through collaborations with a worldwide network of partners. S-MGBs can be obtained from a wide range of structures and physicochemical properties that influence the S-MGB's effect on a given class of target organism. A dominant feature that determines selectivity is access of the S-MGB to the DNA of the target organism which requires passing through the external cell membrane or cell wall. Experiments have shown that S-MGBs containing alkene links in place of an amide are in general most effective against all the infective agents studied but significant activity against some fungi has also been observed in S-MGBs with amidine links. More subtle effects in anti-fungal activity have also been observed relating to the structure of the fungal cell wall. In the case of *M. tuberculososis*, improved selectivity indices were obtained using non-ionic surfactant vesicles in the formulation. Together these results are helpful to identify clusters of S-MGBs that can be optimised to be selective against a given infectious agent.

Keywords: Minor Groove Binder; MGB; Anti-infective

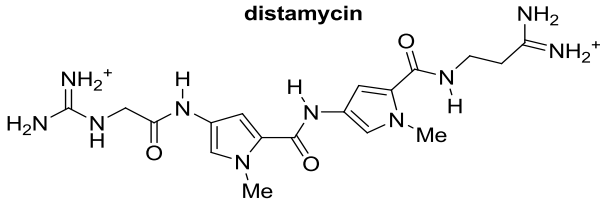
Introduction

Minor Groove Binders (MGBs) are a class of compound that exert their biological effects through binding to the minor groove of DNA.

The MGB drug discovery platform at the University of Strathclyde is based upon the polyamide natural product, distamycin, and the related compound netropsin.



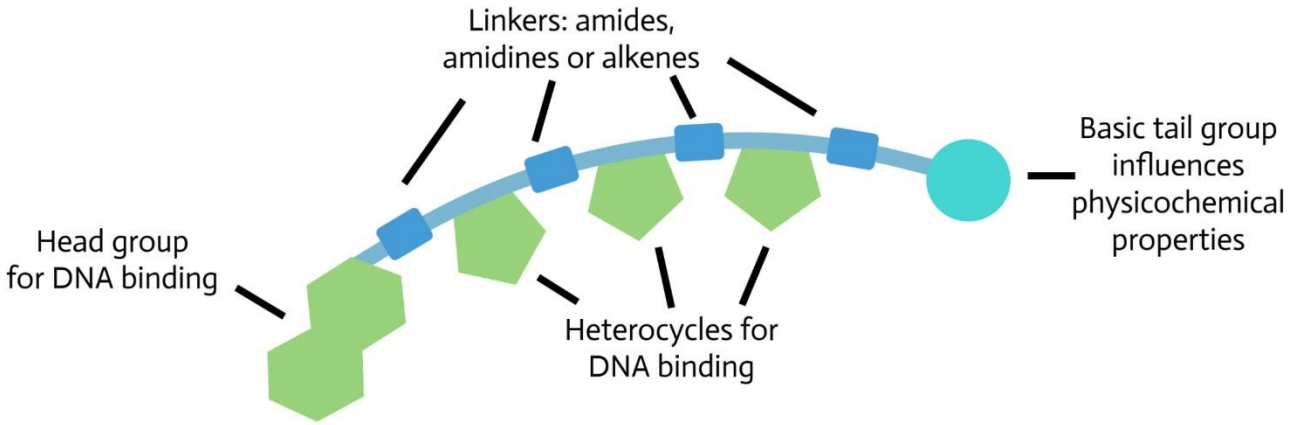
distamycin



netropsin

Analysis of Structure and Design Concept

The structure of distamycin can be conceptually reduced to the following graphic

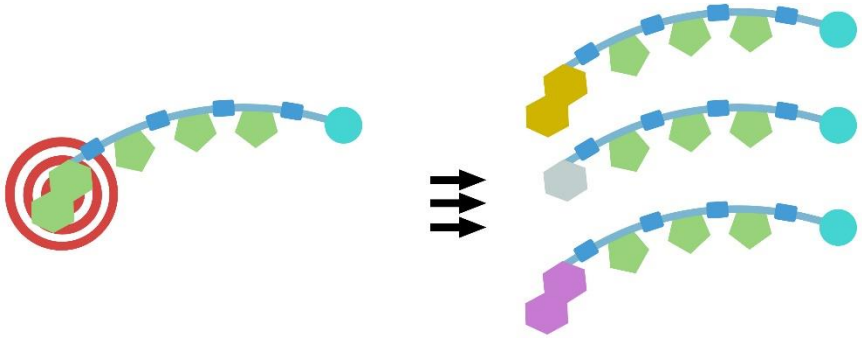


The synthetic strategy for our MGBs involves the sequential coupling of units from the tail group end.

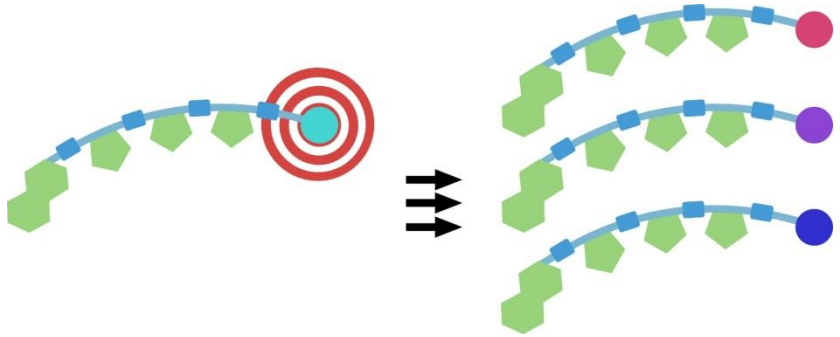
We have assembled a library of over 400 MGBs through systematically varying key structural features of the core MGB structure. These are outlined over the next few slides.

Types of Variation Introduced

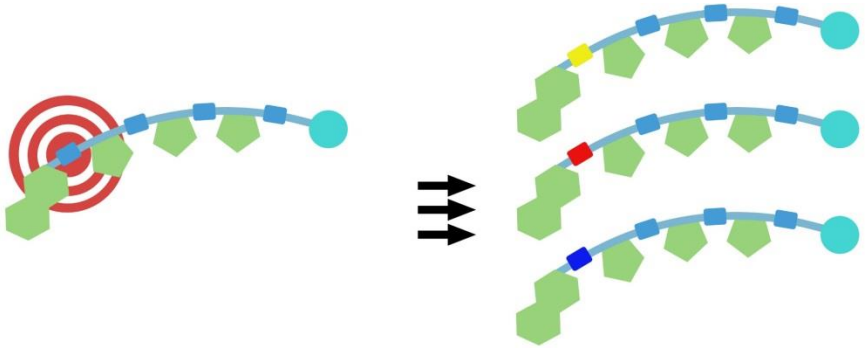
Head Group Diversification



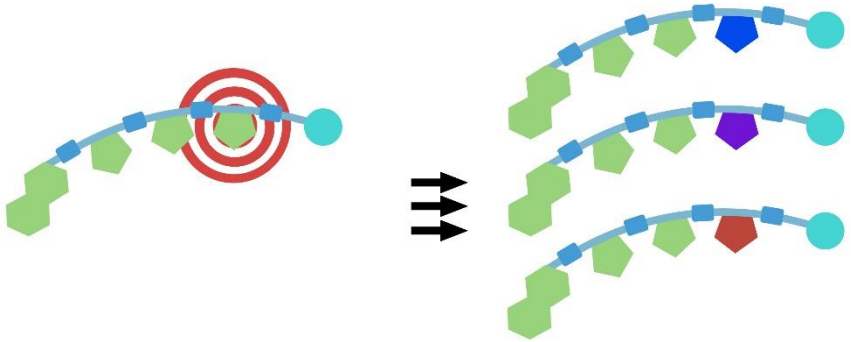
Tail Group Diversification



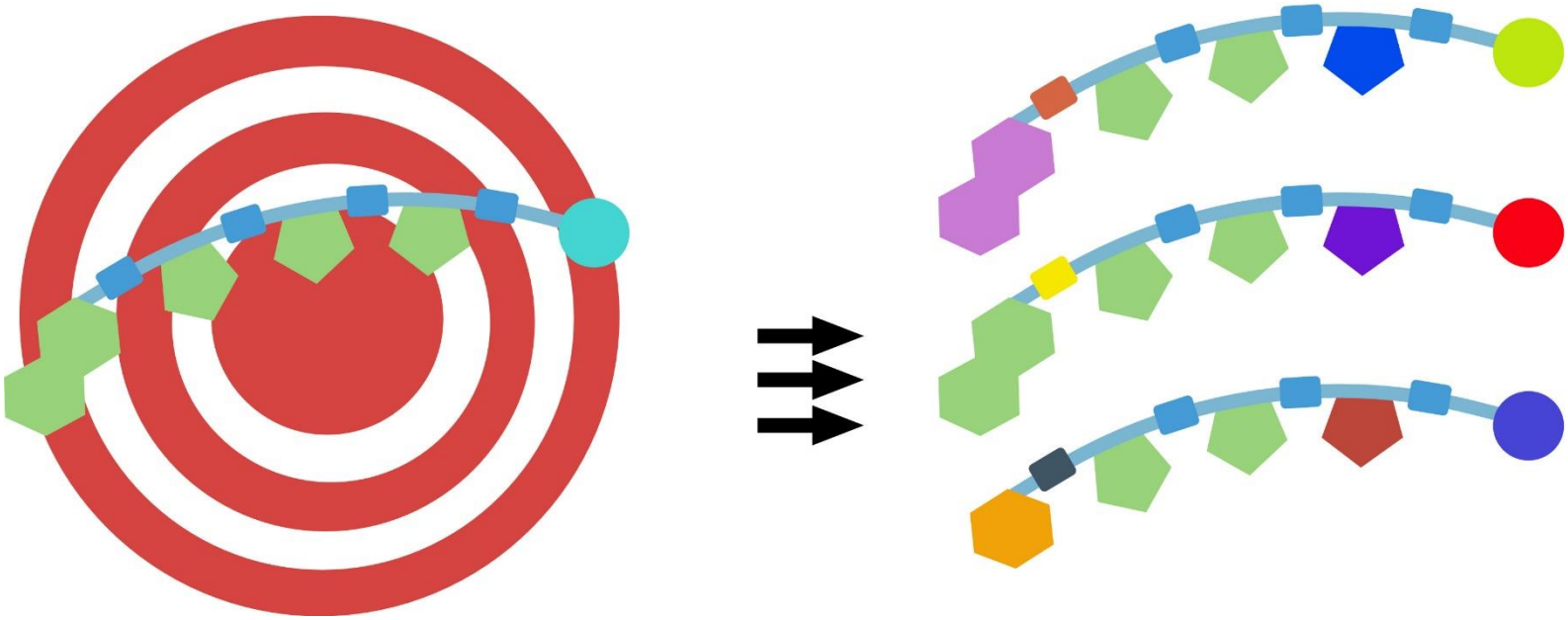
Linker Diversification



Heterocycle Diversification



Multiple Permutations Available



Results and Discussion

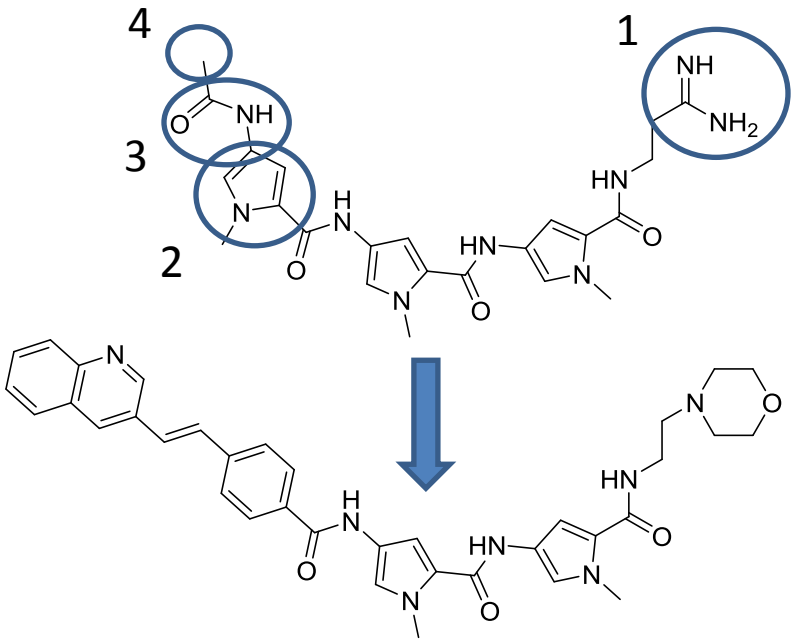
Over a period of many years, our library of MGBs has been evaluated against a wide variety pathogenic organisms. These are outlined below.

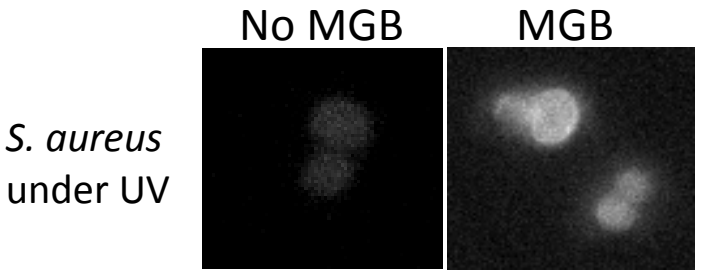
Type of Organism	Organism
Bacteria	*Gram +ve: Staphylococcus aureus, Clostridium difficile*
	Gram –ve: Escherichia coli
	Mycobacteria: Mycobacterium tuberculosis
Parasites	*Trypanosoma brucei brucei*
	Trypanosoma congolense
	Trypanosoma vivax
	Plasmodium facliparum
Fungi	*Candida albicans*
	Cryptococcus neoformans

The following section describes the features of the most active MGBs against each organism, and highlights their significance.

Antibacterial MGBs: Gram-Positive Bacteria

Iain Hunter and Nick Tucker, University of Strathclyde





S. aureus under UV

Divergence from Distamycin:

1. Less basic morpholine tail group
2. Phenyl replaces pyrrolyl
3. Alkene replaces amide head group link
4. Large head group

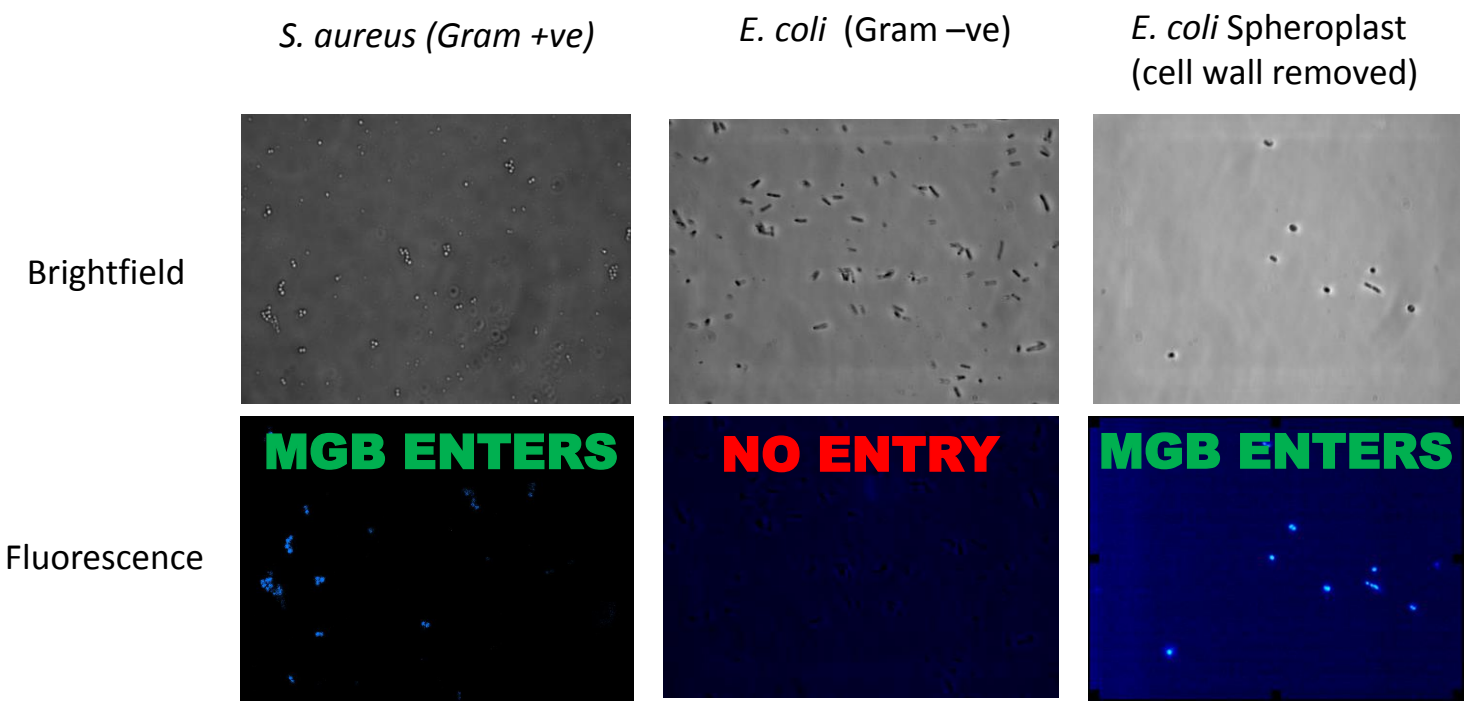
Activity Summary:

1. Sub-µM *in vitro* MICs against many Gram +ves
2. Successful phase I clinical trial for *Clostridium difficile* infections
3. Alkenyl MGBs are fluorescent allowing demonstrable entry into Gram +ve bacterial cells (see panel lower left).

Antibacterial MGBs: Gram-Negative Bacteria

Iain Hunter and Nick Tucker, University of Strathclyde

Typical Gram-positive active MGBs show little Gram-negative activity.
Below shows different cells being treated with a fluorescent MGB

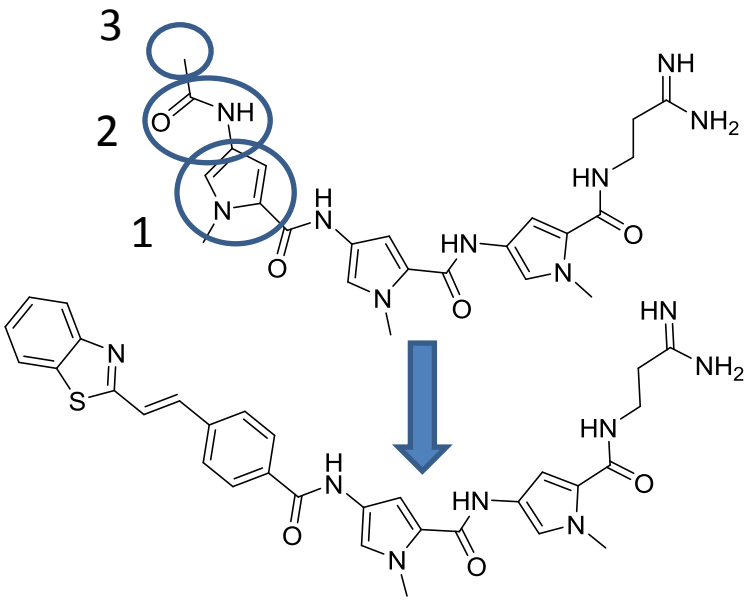


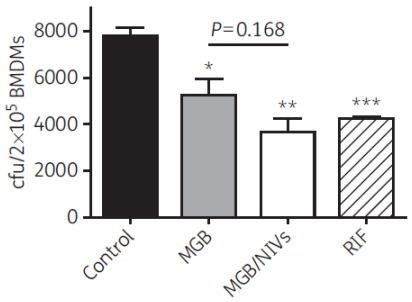
When the outer Gram-negative bacterial cell wall is removed, MGBs can enter.
Lack of Gram-negative activity may be due to poor penetration of bacterial cells.

Antibacterial MGBs: *Mycobacterium tuberculosis*

Reto Guler, University of Cape Town

Hlaka *et al.* (2017) *J Antimicrob Chemother*, doi:10.1093/jac/dkx326





Vesicle MGB formulation (NIVs) achieves activity comparable to that of standard therapy rifampicin

Divergence from Distamycin:

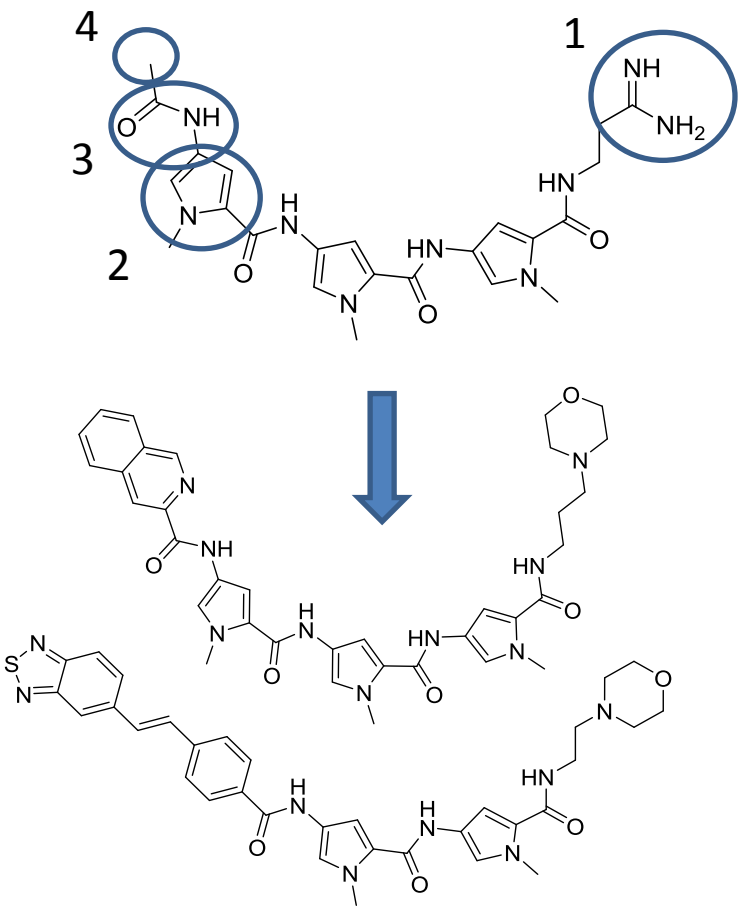
1. Phenyl replaces pyrrolyl
2. Alkene replaces amide head group link
3. Large head group

Activity Summary:

1. Single digit µM intracellular antimycobacterial activity using macrophages
2. Penetrates mammalian cells then bacterial cells to achieve activity
3. Vesicle formulation further enhances activity, presumably through further enhancing cellular penetration
4. No notable toxicity on macrophages

Antiparasitic: *Trypanosoma brucei brucei*

Michael Barrett, University of Glasgow Scott *et al.* (2016) *Eur J Med Chem* doi:10.1016/j.ejmech.2016.03.064

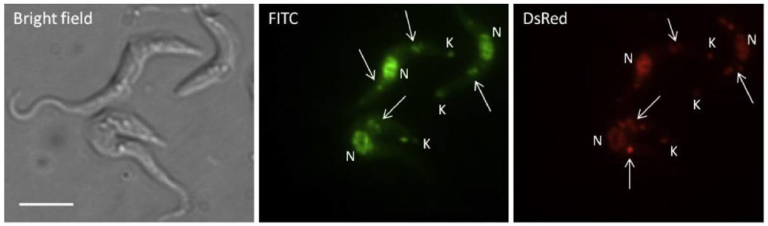


Divergence from Distamycin:

1. Less basic morpholine tail group
2. Phenyl replaces pyrrolyl
3. Alkene replaces amide head group link
4. Large head group

Activity Summary:

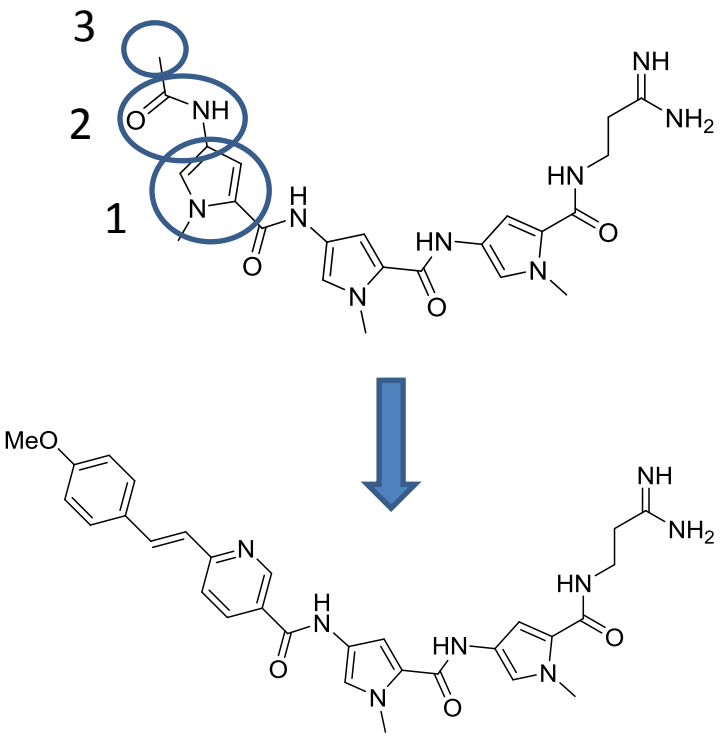
1. IC_{50}s < 40 nM *in vitro*
2. Demonstrable entry into parasites and localisation within DNA-containing organelles.



A fluorescent MGB enters cells and concentrates in DNA-containing organelles (nucleus, N; kinetoplast, K)

Antiparasitic: *Trypanosoma congolense and vivax*

Michael Barrett, University of Glasgow



Divergence from Distamycin:

1. Phenyl/pyridyl replaces pyrrolyl
2. Alkene replaces amide head group link
3. Large head group

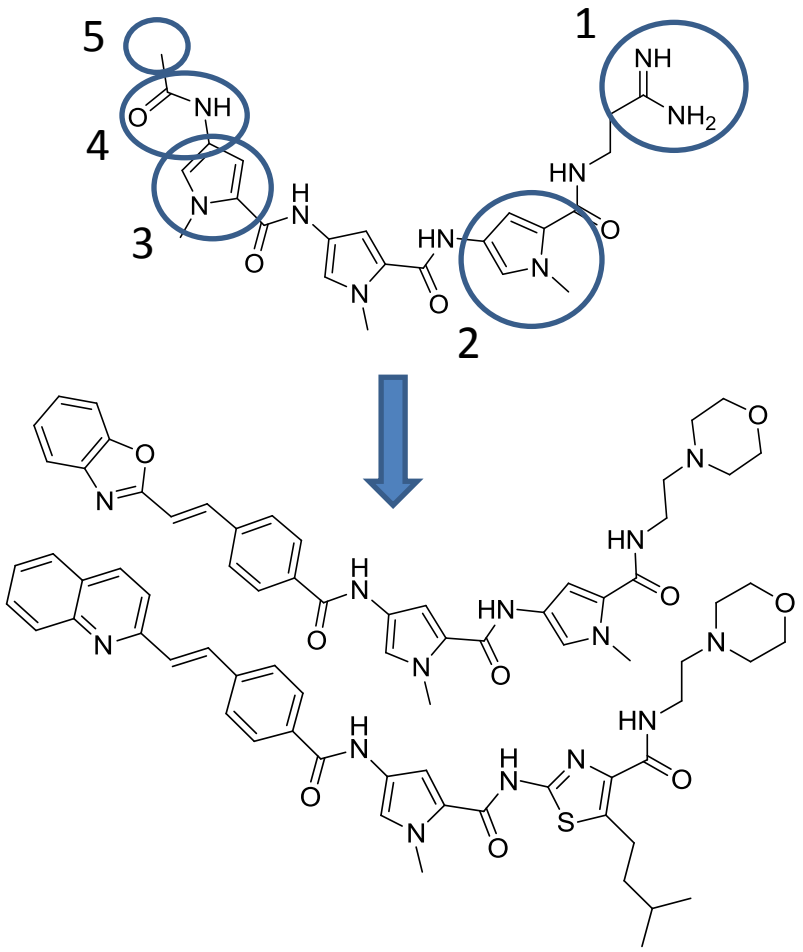
Activity Summary:

1. ~100-300 nM *in vitro* IC_{50}s
2. Selectivity indices of 100-300
3. Curative in *in vivo* mouse models
4. No cross-resistance with common antiparsitics
5. Demonstrable entry into parasites and localisation within DNA-containing organelles (see previous slide).

Antiparasitic: *Plasmodium falciparum*

Vicky Avery, Griffith University

Scott *et al.* (2016) *Bioorg Med Chem Lett* doi:10.1016/j.bmcl.2016.05.039



Divergence from Distamycin:

1. Less basic morpholine tail group
2. Thiazole also tolerated
3. Phenyl replaces pyrrolyl
4. Alkene replaces amide head group link
5. Large head group

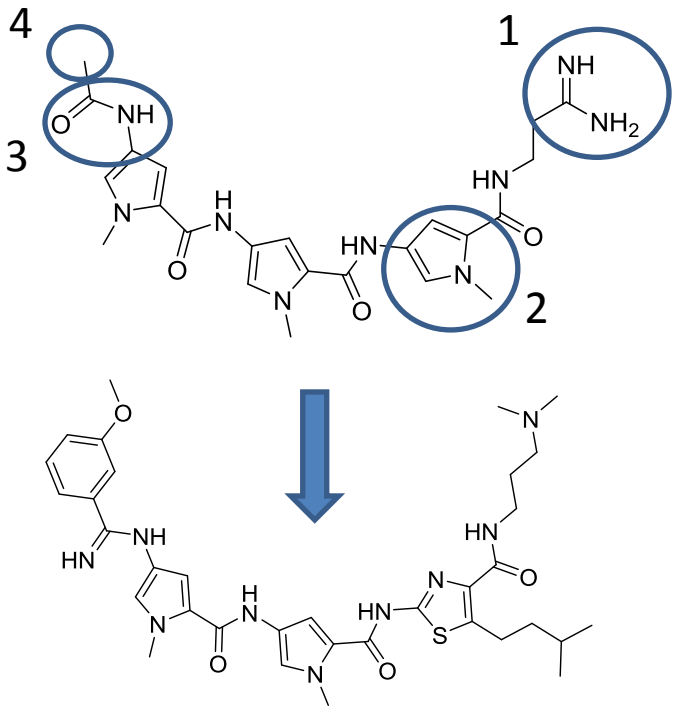
Activity Summary:

1. ~100 nM *in vitro* IC_{50}s
2. Active against chloroquine insensitive strains
3. Selectivity indices >500 against mammalian cells

Antifungal: *Candida albicans* and *Cryptococcus neoformans*

Michael Bromley, University of Manchester

Scott *et al.* (2017) *Eur J Med Chem* doi:10.1016/j.ejmech.2017.05.039



Divergence from Distamycin:

1. Less basic dimethylaminopropyl tail group
2. Thiazolyl replaces pyrrolyl
3. Amidine replaces amide head group link
4. Large head group

Activity Summary:

1. MIC_{70} of 2 mg/mL against *C. neoformans*
2. No observable activity against *C. albicans*

The outer chain mannans of *C. albicans* contain negatively charged phosphodiester links, absent from *C. neoformans*.

The phosphodiester anion could sequester these MGBs through their dicationic nature at physiological pH, thus explaining the lack of activity.

Summary of SAR Across Organisms

Structural Feature	Effect on Organism Selectivity
Large head group	No apparent selectivity, but all active compounds have a larger head group than distamycin
Alkene head group link	Generally increases activity against all organisms, but perhaps not for fungi
Amide head group link	Only effective against *Trypanosoma brucei brucei*
Amidine head group link	Only effective against *Cryptococcus neoformans*
Pyrrole as first heterocycle	Only effective against *Cryptococcus neoformans*
Thiazole as third heterocycle	Effective against *Cryptococcus neoformans and Plasmodium falciparum*
Morpholine tail group	Most active against Gram-positive bacteria, and *Trypanosoma brucei brucei*
Dimethylaminopropyl tail group	Necessary for activity against *Cryptococcus neoformans*
Amidine tail group	Necessary for activity against *Mycobacterium tuberculosis, Trypanosoma congolense and Trypanosoma vivax*

Conclusions

Our MGB platform can provide significant active compounds for a wide range of pathogen organisms

- Phase I clinical trials successfully completed for treatment of *C. difficile*
- MGBs comparable to current treatments, *in vitro*, for *M. tuberculosis* and parasitic organisms

As interacting with DNA is the mechanism of action of our MGBs, DNA binding strength is obviously important for activity; however, cell entry is also important. This explains organism selectivity.

- MGBs significantly active against Gram-positive bacteria are not active against Gram-negative, but removal of the cell wall restores activity
- Selective activity between fungal species can be attributed to failure to penetrate cell wall

We can now begin to design organism specific MGBs

- Amide head group link only effective against *T. brucei brucei*
- Combination of amidine head group link, thiazole as third heterocycle, and dimethylaminopropyl tail group leads to selective *C. neoformans* activity

Acknowledgments

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