The Design and Synthesis of Novel AOX and Cytochrome bc_1 Inhibitors which Act as Phytopathgenic Fungicides

James Misselbrook[a], Luke Young[a], Jonathan Sharp[b] and Anthony L. Moore[a].

[a] Biochemistry, School of Life Sciences, University of Sussex, Falmer, Brighton BN1 9QG, UK.
[b] Research and Development, Agform Limited, Wickham, PO17 5NZ, UK.

The use of fungicides to control foliar crop diseases can be dated back to the early nineteenth century; following the widespread infection of *Phytopthora infestans* (Potato Blight). Potato Blight caused widespread famine across the majority of Western Europe leading to the use of one of the earliest fungicides, the Bordeaux mixture. Today the importance of fungicides can be stressed as easily as it was then; with a growing population of resistant fungi and greater global food demand.

The most prevalent fungal pathogen in the UK is *Septoria tritici*, a major threat to our largest cereal crop, winter wheat. The strobilurin, azoxystrobin, is the most commonly used fungicide for the treatment of this disease. In more recent years, the efficacy of azoxystrobin has diminished due to the emergence of a highly resistant strain of *S. tritici*. Farmers currently rely on using tank mixtures of azoxystrobin and less selective pesticides such as chlorothalonil in order to overcome the fungal resistance. The resistant strain developed by a single site mutation in the cytochrome bc_1 complex, the target site for azoxystrobin. Inhibition of complex III by azoxystrobin has shown to induce the AOX and could therefore be the first step in the resistance mechanism.

It is clear that new classes of fungicides are needed in order to combat resistance and ensure local and global food supplies.

Fungicide Target Sites

The activity of fungicides is often triggered by the suppression of one the mitochondrial respiratory complexes of the fungus. The mitochondria in living organisms, are the site of oxidative phosphorylation, the process that drives the synthesis of ATP. Azoxystrobin is a specific inhibitor of the cytochrome bc_1 complex (Complex III) shown in *Fig 1*. The alternative oxidase located on the matrix side of the inner mitochondrial membrane and branches from the respiratory chain providing an alternative pathway for respiration.

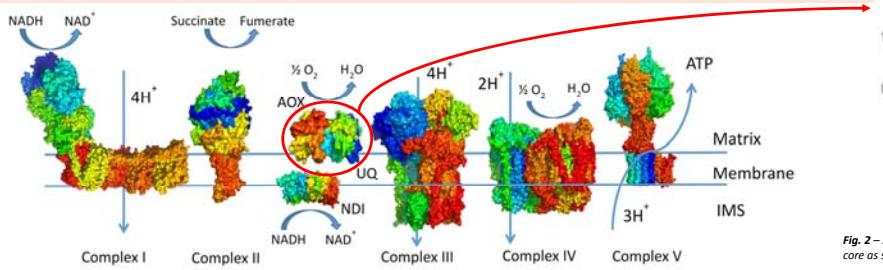

Fig. 1 *– Mitochondrial respiratory chain. Displaying respiratory complexes I-V and the alternative oxidase (AOX).*

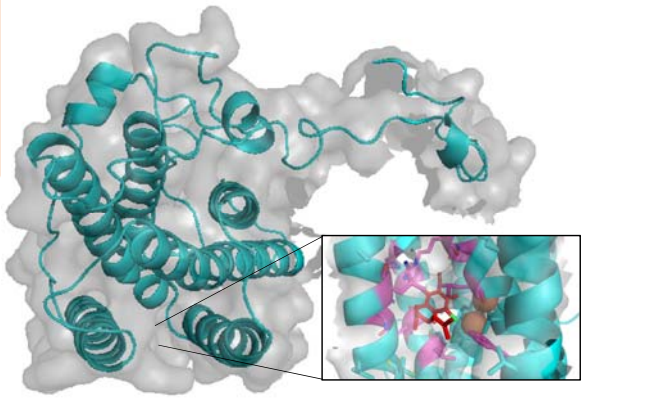

Fig. 2 *– S. tritici AOX modelled on the tryponsoma brucei brucei crystal structure* ***Inset****: Colletochlorin B bound active site showing di iron core as spheres and important residues in magenta.*

AOX and Fungicide Resistance

The mechanism to which fungi, such as *Septoria tritici*, become resistant is controversial and thought to be influenced by a number of factors. Alternative respiration has been reported to be one of the major triggers leading to resistance in *Septoria tritici*[1]. It has been shown that inhibition of the cytochrome bc_1 complex results in the induction of the AOX[2], and could be a dominant resistance mechanism. It is therefore essential to find an inhibitor that will bind to the active sites of both the cytochrome bc_1 complex and AOX.

Fungicide Design

In order to synthesise an effective fungicide a number of physical and structural features need to be considered. The naturally occurring colletochlorin-type inhibitors have been shown[3] to inhibit both the AOX and cytochrome bc_1 complex. These compounds will provide a framework to base new novel fungicides around.

A recent study[4] on the AOX responsible for African sleeping sickness (*Tryoansoma brucei brucei*, TAO) has attempted to establish a pharmacaphore for AOX binding site. With very little information on the *S.tritici* AOX binding site, comparisons can be made with the TAO binding site (Fig. 3 and Fig 4.) in order to design a specific inhibitor. Dose response data (Table 1) indicates the efficacy of existing TAO inhibitors against both the fungal and TAO alternative oxidases.

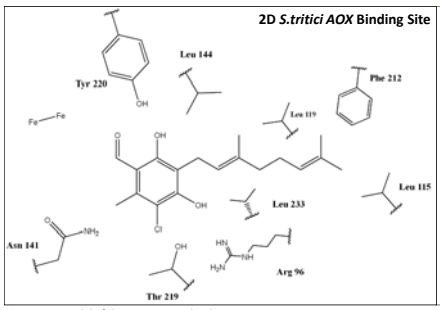

Fig. 3 *– 2D model of the S. tritici AOX binding site*

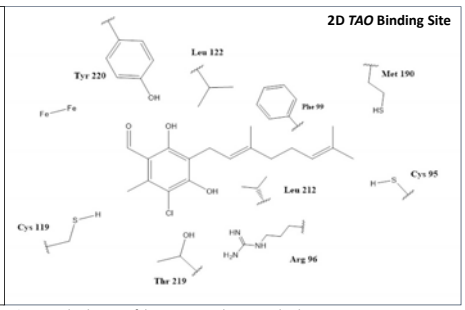

Fig. 4 *– 2D binding site of the trypanosma brucei AOX binding site*

	S. tritci AOX	TAO AOX*
Inhibitor	IC-50	
SHAM	20mM	0.23 µM
Octyl Gallate	2.3µM	0.13 µM
Ascofuranone	0.90µM	7.4nM
Ascochlorin	0.18µM	24nM
Colletochlorin B	0.80µM	7.5nM

Table 1 *– Dose response data from current AOX and cytochrome bc_1 complex inhibitors.*

Synthesis of New Fungicides

So far, synthesis has focussed on the isoprenoid terminal end of the colletochlorin B structure. A very simple and effective tail reaction scheme (Fig. 7) has provided the platform for a vast number of novel inhibitors via a simple reductive amination step. The head group can be synthesised by two very facile steps (Fig. 6) and can be linked to the tail group created in Fig. 7. Manipulation of the properties of the tail group should encourage positive binding data, due to the presence of the Phe-212 residue in S.tritici (Fig. 3).

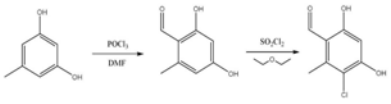

Fig. 5 *– Head group synthesis for Colletochlorin B type inhibitors*

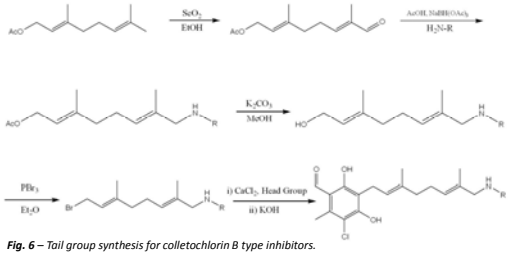

Fig. 6 *– Tail group synthesis for colletochlorin B type inhibitors.*

Future Work

To better understand the fungal AOX inhibitor binding site, synthesis will include structural changes to both the head group and isoprenoid chain. This will provide informative comparisons when it comes to enzyme kinetic studies. The new compounds will be analysed via a number of different assay systems, against both the AOX and the cytochrome bc_1 complex.

References:
(1) Ziogas, B. N., Baldwin, B. C. & Young, J. E. Alternative respiration: a biochemical mechanism of resistance to azoxystrobin (ICIA 5504) in Septoria tritici. Pesticide Science 50, 28-34 (1997).
(2) Wood, P. M. & Hollomon, D. W. A critical evaluation of the role of alternative oxidase in the performance of strobilurin and related fungicides acting at the Qo site of complex III. Pest management science 59, 499-511 (2003).
(3) Moore, A.L., Albury, M.S., Young, L.E. and Elliott, C., The University Of Sussex, 2013. Compounds for use as inhibitors of alternative oxidase or cytochrome bc1 complex. U.S. Patent Application 14/397,164.
(4) Saimoto, H., Kido, Y., Haga, Y., Sakamoto, K. & Kita, K. Pharmacophore identification of ascofuranone, potent inhibitor of cyanide-insensitive alternative oxidase of Trypanosoma brucei. Journal of biochemistry 153, 267-273 (2013).