

AOX Inhibitors

Evaluation and Licensing Opportunities

For further information on this technology and evaluation / licensing opportunities please contact:

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Patent Literature

US 10,851,047 and 11,565,996 and numerous corresponding patents

Broad crop protection applications (pathogens & herbicide)

Overcomes emerging strobilurin resistance

Breakthrough suite of chemistry targeting AOX

Rational design based on fundamental active site studies

Fungal infections presently destroy at least 125 million tonnes of the top five food crops (rice, wheat, maize, potatoes and soybeans) each year. The damage caused by fungal pathogens to rice, wheat and maize alone costs global agriculture \$60 billion per year. While strobilurins are mitigating this loss, the increasing resistance to this class of fungicides is an extremely worrying trend. Now Professor Tony Moore and colleagues at Sussex University have developed a novel class of antifungal agents based on their detailed proprietary insights on AOX inhibitors. AOX is an alternative respiratory pathway which becomes activated when the organism becomes stressed. Their insights and fundamental work on AOX facilitate the development of AOX inhibitors which will enable the crop protection industry to both overcome the problem of strobilurin resistance and deliver robust fungal control solutions. In addition, AOX inhibitors also have the potential to be used as anti-microbial agents and herbicides by targeting the specific AOX protein of the target organism.

The Moore lab makes Sussex a global centre of excellence for AOX research having studied the structure and function of the alternative oxidase (AOX) for more than 40 years. The detailed structural knowledge of different AOX binding pockets and hydrophobic cavity configuration enables the rational design of compounds targeted at specific AOX proteins. AOX is widespread in fungal and microbial pathogens, as well as in plants, and Professor Moore's work enables the rational design of compounds inhibiting a specific AOX. Using this knowledge it is possible to create compounds that can be used as fungicides, anti-microbial compounds and herbicides opening up the possibility to design a wide range of crop protection chemicals.

Many fungicides' mode of action is to block their target organism's ability to respire; azoxystrobin and other strobilurins inhibit mitochondrial respiration by blocking the electron transport pathway. They bind at the quinol outer binding site of the cytochrome bc₁ complex (complex III in figure 1 below), where ubiquinone (coenzyme Q10) would normally bind thereby inhibiting the electron transport pathway and production of ATP. The compounds causing this mode of action are called Quinone Outside Inhibitors ("QoI"). Strobilurins have proven to be very effective fungicides but now resistance to this class of fungicides is increasingly occurring, as fungal populations have the ability to develop resistance to QoI inhibitors by using alternative pathways to produce ATP. AOX enables an alternative respiratory pathway that allows fungi to bypass the site of action of QoI fungicides thereby enabling fungi to continue to respire and grow.

Tony Moore's team has studied the structure (see Figure 2 below) of the alternative oxidase allowing them to develop highly specific AOX compounds which prevent AOX from functioning. This structural analysis has resulted in the design of very specific inhibitors. Crucially, these inhibitors do not interact with or inhibit any other proteins. It should be noted that AOX is not found in mammalian, vertebrate or insect tissues. Professor Moore and his team are continuing to investigate the structure, function, and mechanism of action of AOX and are using this information, in particular, to design and synthesise a number of AOX specific compounds, which could be used as phytopathogenic agents.

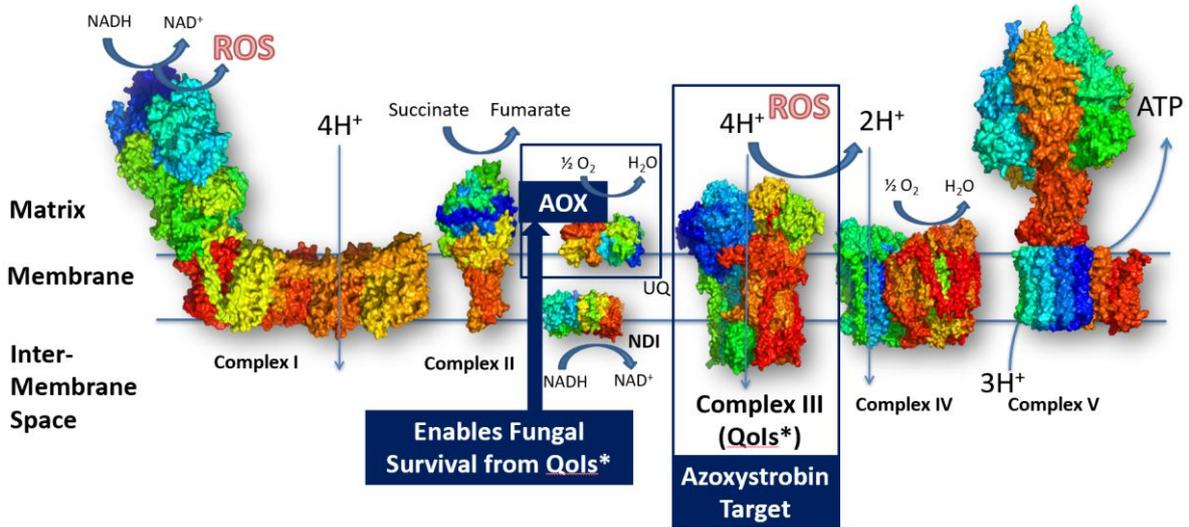


Figure 1: Complex III (cytochrome bc_1 complex) is the Azoxystrobin fungicide target. Blocking Complex III = enhanced reactive-oxygen species (ROS) production leading to induction of AOX activity. In the presence of AOX, Complex I becomes the only source of ATP generation albeit at a significantly reduced rate. The Citric acid cycle still turns over as AOX enables continued activity of Complex I, II and NDI thereby facilitating fungal survival. Inhibiting AOX (in the presence of a CIII inhibitor) has the potential to prevent electron transport and ATP production thereby inhibiting fungal growth

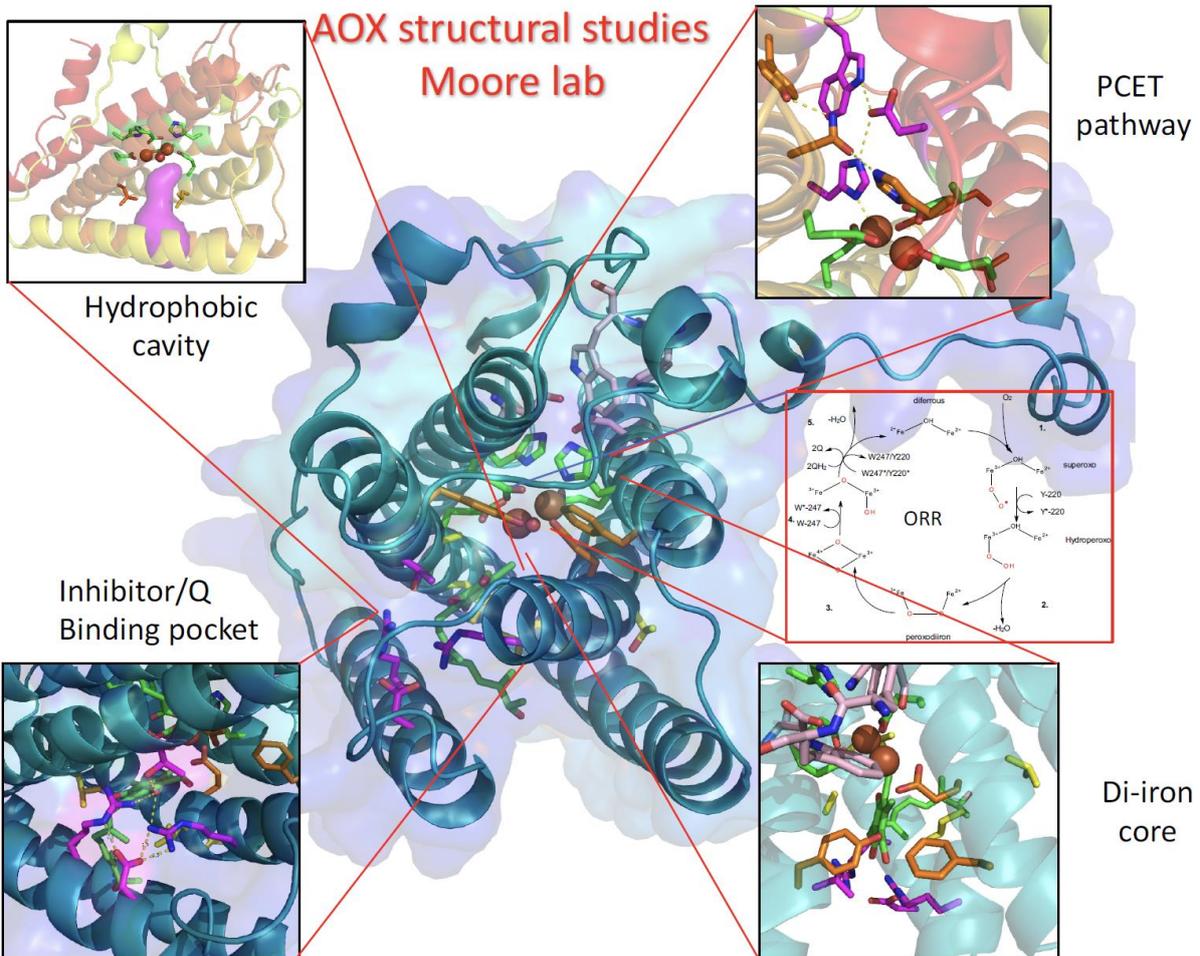


Figure 2: A key challenge in understanding the structure-function relationship of AOX has been the identification of its substrate and inhibitor-binding site and its mechanism of action, facilitating the rational design of compounds that are specifically targeted to the alternative oxidase. The quinone-binding site of the AOX is a suitable and promising target in the treatment of pathogens.

The detailed structural knowledge of the nature of the protein-ligand interaction has allowed the design and synthesis of a library of novel AOX inhibitors. Through the fundamental research this allows the rational design for specific AOX targets ranging from fungi to microbes and plants. The team's work has already resulted in the generation of a suite of compounds that have the capacity to act as phytopathogenic agents specifically targeted at the AOX, as well as the ability to design and synthesize further chemicals targeting AOX. In addition, it is also possible to design compounds like ascochlorin which can inhibit both AOX and the bc1 complex, which will lead to a completely novel class of fungicides which will be independent of strobilurins. Further development work is underway in Professor Moore's lab to make such compounds much more potent and cheaper to manufacture.

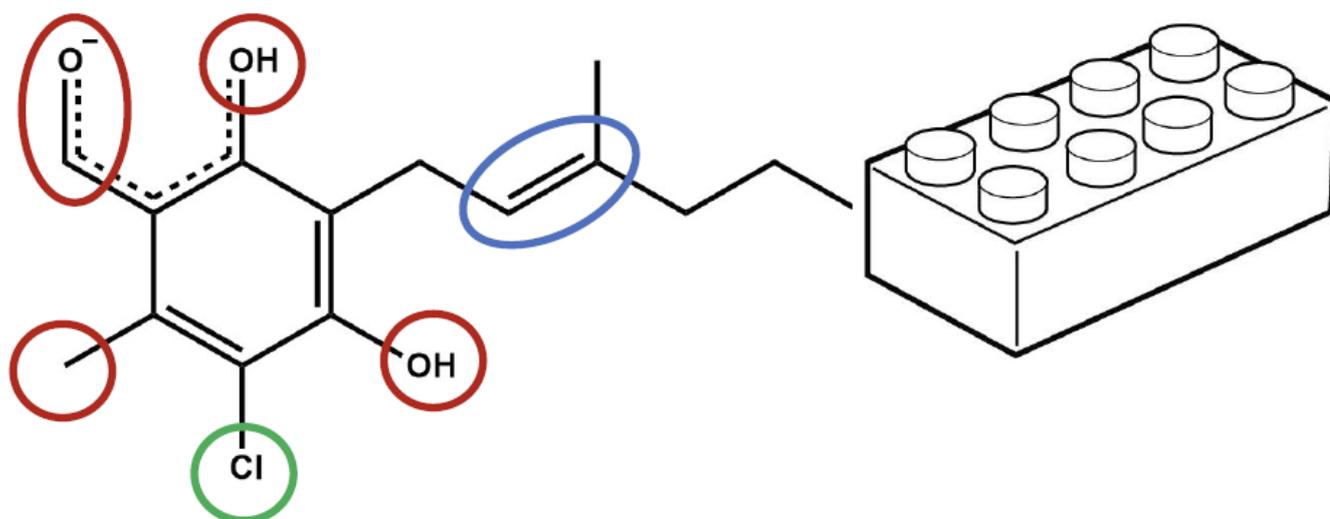


Figure 3: Outline design for AOX inhibitors

Hydroxyl groups - Hydrogen bonding acceptors/donators essential.

Methyl group - potential position for fungal selectivity combined with tail group

Halogen - Halogen preferred but not essential

Aldehyde – Essential to inhibitor binding with potential delocalisation- H-bond and/or cation interactions

Isoprenoid - orientates the tail into the correct position

A number of highly specific AOX inhibitors have already been synthesized and they follow the general design (which can be modified depending on exact 3D structure of the specific target AOX) as outlined in Figure 3 above. Different compounds have been tested on membrane-bound and purified AOX protein expressed in a haem-deficient strain of *E. coli*. To date, a library of over 20 compounds has been established which display characteristics of being useful phytopathogenic inhibitors. Figure 4 below shows an example of three compounds on plate tests with 5 different fungal species.

The wide range of compounds outlined in Figure 3 above is protected by strong, recently granted patents in numerous territories. There are two main patent families with claims on this wide range of compounds, one family covers the compounds and their use for a range of applications and the second family specifically covers their use as fungicides with strobilurins.

The University of Sussex team has demonstrated that fungal plant pathogens such as *Septoria tritici*, causing major leaf spot diseases in wheat, and the wheat "Take-all" fungus (*Gaeumannomyces graminis* var. *tritici*) have the capacity to express AOX when pathogens are treated with respiratory inhibitors such as the strobilurins. Such treatments may result in the development of 'strobilurin-resistant respiratory pathways' facilitated by AOX thereby potentially accounting for the varying efficacy of strobilurin fungicides.

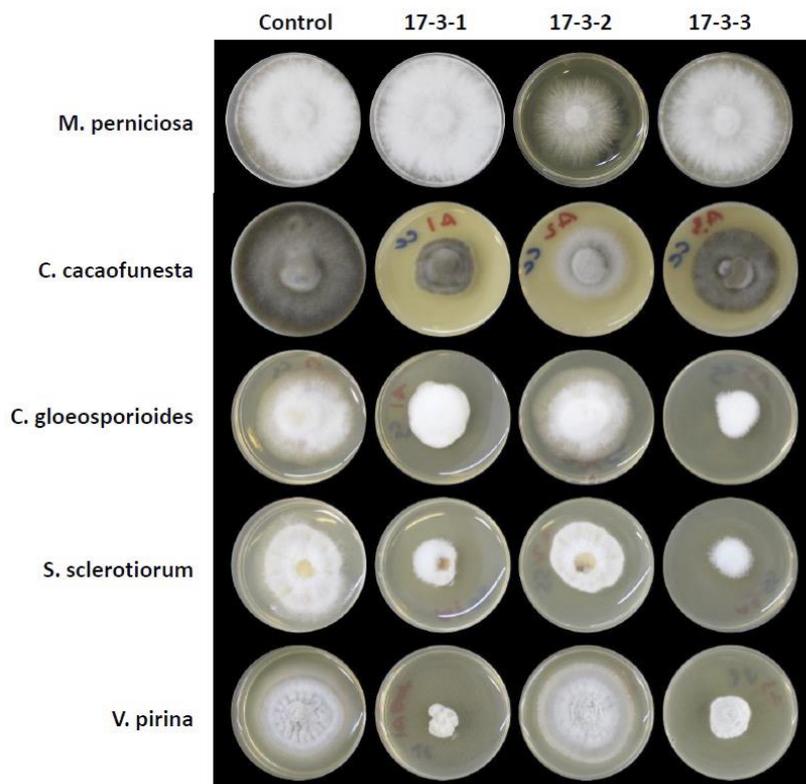


Figure 4: Plate assay system with 3 AOX fungicides (1 μ M) tested against 5 fungal pathogens: *M. Perniciosa* (witches' broom disease), *C. cacaofunesta* (wilt disease), *C. gloeosporioides* (anthracnose), *S. sclerotium* (white mold) and *V. pirina* (scab disease)

All this fundamental groundwork described above provides a platform to use this technology for commercial exploitation through the development of specific AOX inhibitors for a wide variety of applications in agrochemical markets. This provides a breakthrough solution to prevent fungicide resistance, develop new fungicides, anti-microbial agents and herbicides and thereby increase crop yields worldwide. The AOX inhibitor can either be designed as a dual-mode inhibitor targeting both Complex III and AOX or alternatively an AOX-only inhibitor which can be used in conjunction with a Complex III targeting fungicide.

PBL is working with the University of Sussex and AlternOx Scientific Ltd (AlternOx) to engage with development partners to design, test and commercialise novel fungicides, anti-microbial compounds and herbicides through the rational design of targeted AOX inhibitors. AlternOx is a newly established bio-pharma company (and spin-out of University of Sussex) focused on the exploitation of novel and proprietary inhibitors of AOX to treat a wide range of multi-drug and multi compound resistant species of fungi that now threaten human health.

We encourage interested parties to contact us to discuss possible avenues either to access the range of chemistry already developed at the University of Sussex and/or to discuss the design of new AOX inhibitors based on the expertise of Professor Moore and AlternOx. In addition, there is also the possibility of collaborating with Professor Moore's laboratory and/or AlternOx to design, synthesize and/or test AOX inhibitors.

References:

Szibor, M *et al.* (2022) Targeting the alternative oxidase (AOX) for human health and food security, a pharmaceutical and agrochemical target or a rescue mechanism? *Biochemical Journal*; **479**: 1337-1359. <https://doi.org/10.1042/BCJ20180192>