# Novel inhibitors of the carotenoid cleavage dioxygenase enzyme family

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## 1 Introduction

- Carotenoid cleavage dioxygenases (CCDs) are iron dependent dioxygenase enzymes which control the break down carotenoid signalling molecules in plants and animals to give apocarotenoid signalling molecules involved in many important biosynthetic pathways.
- The breakdown products are involved in several important biological processes in plants. For example, strigolactone is an apocarotenoid involved in shoot branching, whereas others are involved in seed germination and controlling oxidative damage.
- Different CCDs can cleave apocarotenoids at different positions along the long chain. *LeCCD1* cleaves at the 9,10 position, whereas *LeNCED1* cleaves at the 11,12 position.

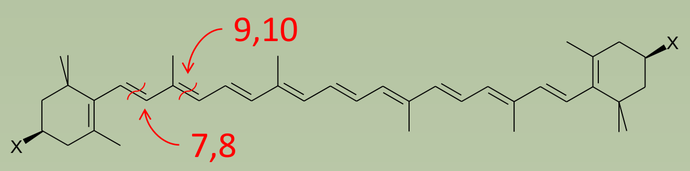


Figure 1 – A typical carotenoid. X represents a different substituent depending upon the carotenoid

- In order to understand the pathways that lead to the production of these apocarotenoids and to understand the effects CCDs have on biological systems, a chemical genetics approach is required using effective inhibitors against the CCD enzymes.

## 3 Results

- Through the use of $C^{13}$, $H^{1}$ and EIMS it has been possible to confirm the synthesis of all five target inhibitors.
- D15, D20, D21 & B2 were all tested against the strongest inhibitor, D2.
- Qualitatively, inhibition can be seen from the colour change on the experimental plate. As substrate (β-apo-8'-carotenal) is broken down there is a colour change from orange to yellow:

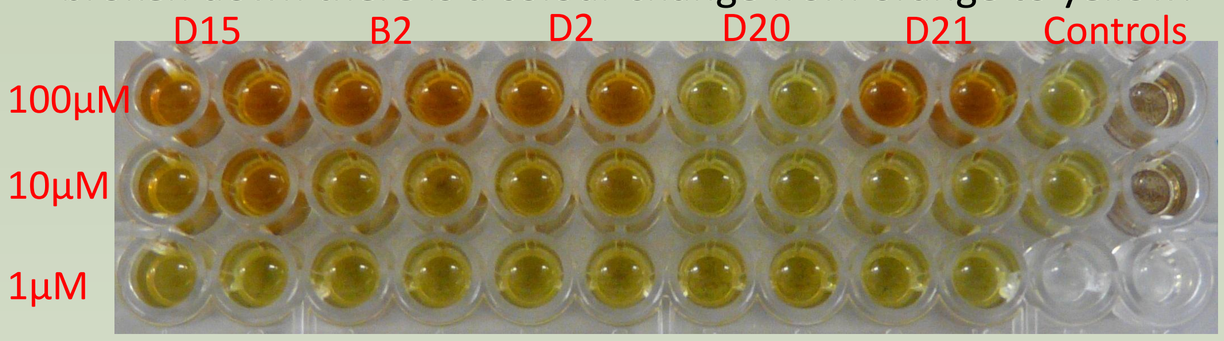


Figure 3 – The colour change seen on the experimental plate after incubation for one hour.

- More detailed data is given by the rate of change in absorbance vs. time. A decrease in absorbance indicates substrate being utilised, with the closer the gradient of the slope being to that of the control, the worse the inhibitor.

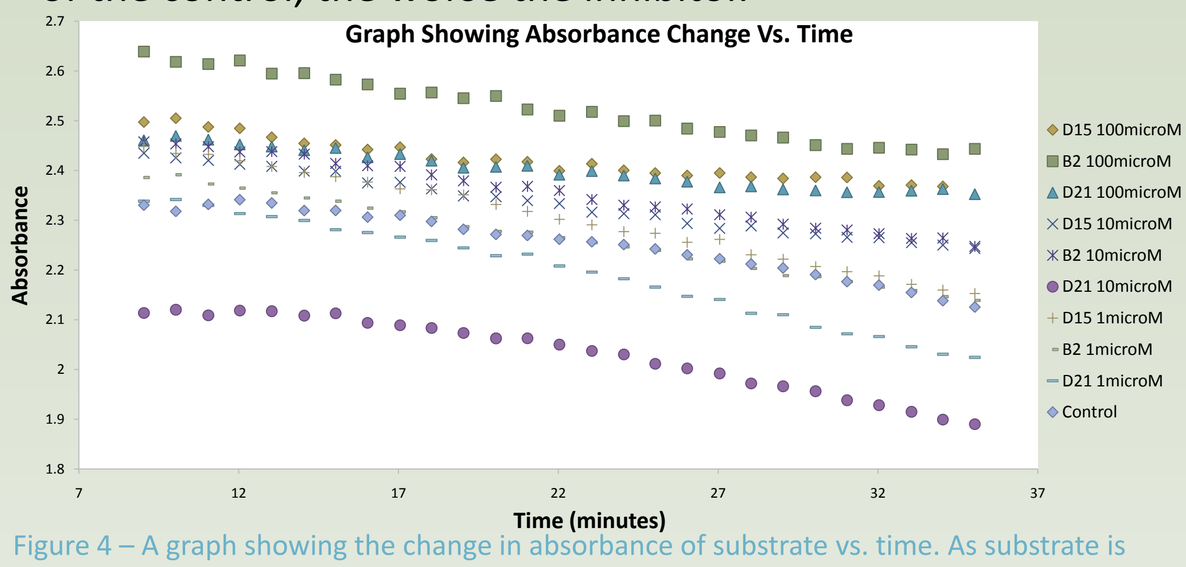


Figure 4 – A graph showing the change in absorbance of substrate vs. time. As substrate is converted to product, the absorbance decreases.

- From figure 4 it can be seem that at 100µM D15, B2 and D21 all inhibit. At 10µM the gradient of D21 and B2 moves close to that of the inhibitor, indicating no inhibition.
- For D15, the slope becomes close to that of the control at 1µM.

## 2 Aims & Methods

- The aim of the project was to synthesise and assay five new members of the CCD enzyme inhibitor family against *LeCCD1*.
- The inhibitors were synthesised via the procedure below.

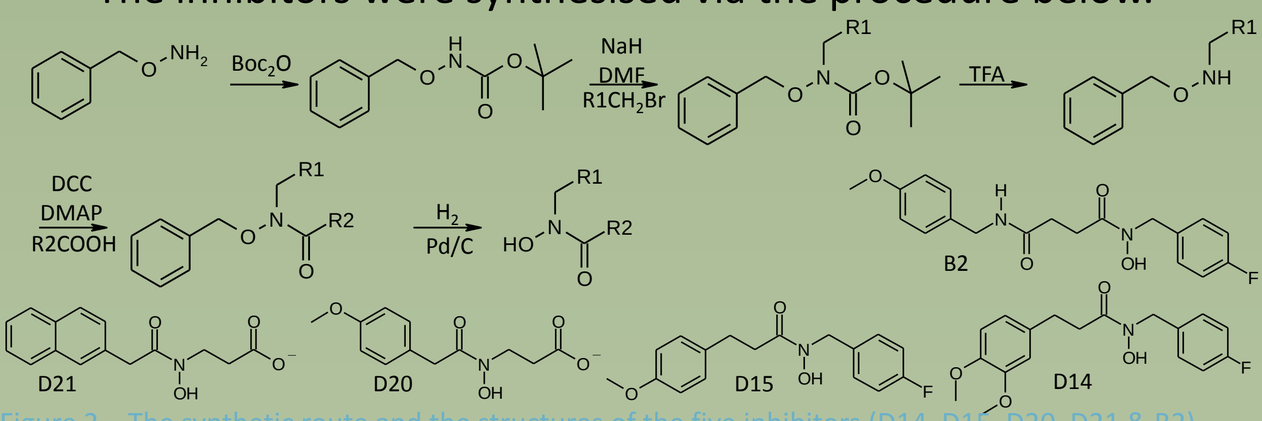


Figure 2 – The synthetic route and the structures of the five inhibitors (D14, D15, D20, D21 & B2)

- Testing *in vivo* to study effects on seed germination and shoot branching on *arabidopsis* was done by *Warwick HRI*.
- *In vitro* biological assays were done on *E.coli* containing over expressed *LeCCD1* with different inhibitor concentrations (100, 10 &1 µM) and monitoring the absorbance at 485nm over a fixed period of time.

## 4 Conclusions

- From the results it can be concluded that D15 is the most effective of the new inhibitors against *LeCCD1*.
- D20 showed no inhibition due to solubility issues.
- D21 and B2 showed inhibition against the 9,10 cleavage enzyme at high concentrations. However, B2 was designed to be effective against *LeNCED1* and D21 against the latter stages of strigolactone production, hence neither was expected to be potent against *LeCCD1.*

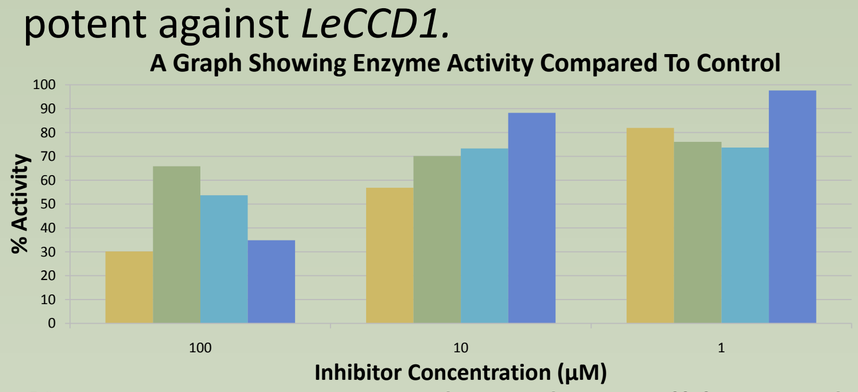


Figure 5 – A Graph showing the percentage activity compared to the control at 100, 10 & 1 M for D15, D2, D21 & B2. I.e. Control = 100% activity.

- Since assays were conducted on cell lysate due to the high instability of the enzyme, detailed kinetic studies are not possible. However $IC_{50}$ values showing the concentration of inhibitor at which 50% inhibition is achieved can calculated.
- $IC_{50}$ values were calculated at ≈10µM for D15, ≈100µM for B2 and ≈100µM for D21. Lower $IC_{50}$'s suggest a stronger inhibitor.
- Seed germination assays *in vivo* proved negative due to the toxicity of the inhibitors on *arabidopsis*.

## 5 URSS Experience

- The URSS scheme has provided me with an invaluable insight into the world of research chemistry.
- Working full time in the laboratory has allowed me to both hone skills I already possess and learn new skills, such as working with biological materials on scales larger than in normal undergraduate labs.
- Finally, by working in a group with other researchers I have been able to develop transferable skills such as presentation, team working and organisational skills.
- Thanks to Mark Ahmad, Darren Braddick, Sandeep Sandhu, Tim Bugg, Martin Seargent, Ann Smith and the Bugg group for their help on the project.

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