Institution: Queen's University, Belfast

Unit of Assessment: 8

Title of case study: Biocatalysts for industrial and medical applications

1. Summary of the impact (indicative maximum 100 words)

Queen's University Belfast has developed a number of biocatalytic processes for the production of pharmaceutical intermediates which have been applied commercially. The most significant involved Vernakalant, a new drug for treatment of the most common form of irregular heartbeat, now available in the EU, and currently awaiting approval in the USA and Canada. In addition, QUB has sold £300,000 worth of bioproducts and through the collaborations with Almac Sciences facilitated the initiation of their biocatalysis business which currently is a multi-million revenue earner for Almac Sciences and employs 30 staff, including 15 PhD graduates from the Queen's group.

2. Underpinning research (indicative maximum 500 words)

The research at Queen's supervised by Boyd (now Emeritus Professor) and Stevenson has led to an understanding of the remarkable potential of a range of biocatalysts which produce single enantiomer polyoxygenated products from aromatic substrates. From 1993, Boyd, in collaboration with Dalton (Warwick), who supplied the initial enzymes (toluene dioxygenase mutant strains), developed the synthesis of the *cis*-dihydrodiols from monosubstituted benzenes which were used as intermediates for the synthesis of Vernakalant (Figure 1).

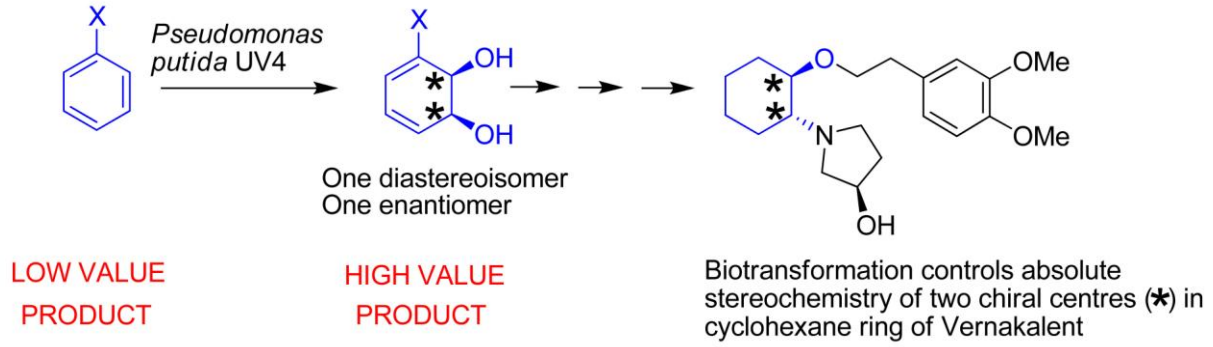

Figure 1 Reaction scheme for the formation of *cis*-dihydrodiols from monosubstituted aromatics leading to the formation of Vernakalant

Importantly, the ability to translate the fundamental research into a commercial success, was due to the Queen's group enabling the determination of structure, configuration and enantiopurity of these novel bioproducts (References 1 and 2 *in section 3*). Through this knowledge, the scope and diversity of the bioproducts produced has been expanded significantly and the limitations of the substrates able to be biotransformed alleviated by the use of manipulation of the biocatalyst employed. The identification of the latter was critical and the Queen's group led the search for new enzymes through development of recombinant strains within Biological Sciences in Queen's (Kulakov and Allen) as well as with Gibson (University of Iowa) developing naphthalene and biphenyl dioxygenases. This research included the first example of site-directed mutagenesis being used to modify dioxygenase regio- and stereo-selectivity (Reference 3 *in section 3*). From these new catalytic systems, previously unknown families of enantiopure metabolites with novel structures, such as sulfoxide diols, arene-derived triols and tetrols, cyclohexenone *cis*-diols, arene oxides and hydrates, with virtually all the products of the biocatalytic reactions being single

enantiomers, were developed (Reference 4 *in section 3*). Furthermore, empirical models were devised to allow prediction of the preferred products formed and the absolute configurations of new bio-products. In addition, this research has contributed to an understanding of why polycyclic aromatic hydrocarbon (PAH) pollutants are human carcinogens; mammalian redox enzymes (monooxygenases) play a key role in turning PAHs into arene oxide and diol epoxide metabolites that interfere with DNA replication.

From the knowledge and fundamental understanding of the biocatalytic pathways, and the manipulation of the conditions used for the reaction and separation processes, as well as the analytical techniques established, commercialisation of the bioproducts has been achieved. This understanding has led to the utilisation of a wide range of substrates which had previously been intractable and, therefore, the selective and efficient production of a large number of desirable pharmaceuticals and fine chemicals in an economic process. Applications of its enantiopure bioproducts have been made in the synthesis of natural products including pericosines (see reference 5 *in section 3*) and epibatidine, unnatural products, such as carbasugars and chiral ligands, for example, bipyridines (see reference 6 *in section 3*), aminoalcohols and phosphines/phosphine oxides.

3. References to the research (indicative maximum of six references) * signify the references which best indicate the quality of the underpinning research

*1. Boyd, D. R.; Sharma, N. D.; Barr, S. A.; Dalton, H.; Chima, J.; Whited, G.; Seemayer, R., Chemoenzymatic Synthesis of the 2,3-*Cis*-Dihydrodiol and 3,4-*Cis*-Dihydrodiol Enantiomers of Monosubstituted Benzenes. J. Am. Chem. Soc. 1994, 116, (3), 1147-1148. (DOI: 10.1021/ja00082a053)

2. Allen, C. C. R.; Boyd, D. R.; Dalton, H.; Sharma, N. D.; Brannigan, I.; Kerley, N. A.; Sheldrake, G. N.; Taylor, S. C., Enantioselective bacterial biotransformation routes to *cis*-diol metabolites of monosubstituted benzenes, naphthalene and benzocycloalkenes of either absolute configuration. J. Chem. Soc., Chem. Commun. 1995, (2), 117-118. (DOI: 10.1039/c39950000117)

3. Parales, R. E.; Resnick, S. M.; Yu, C. L.; Boyd, D. R.; Sharma, N. D.; Gibson, D. T., Regioselectivity and enantioselectivity of naphthalene dioxygenase during arene *cis*-dihydroxylation: Control by phenylalanine 352 in the alpha subunit. J. Bacteriol. 2000, 182, (19), 5495-5504. (DOI: 10.1128/JB.182.19.5495-5504.2000))

*4. Boyd, D. R.; Sharma, N. D.; Malone, J. F.; Allen, C.C.R . New families of enantiopure cyclohexenone-*cis*-diol, o-quinol dimer and hydrate metabolites from dioxygenase-catalysed dihydroxylation of phenols, Chem. Commun.,2009, 3633-3635 (DOI: 10.1039/b905940g).

*5. Boyd, D. R.; Sharma, N.D.; Acaru, C.; Malone, J.F.; O'Dowd, C.R. Allen,; C. C.R.; Stevenson, P.J., Chemoenzymatic synthesis of carbasugars (+)-pericosines A-C from diverse aromatic *cis*- dihydrodiol precursors, Org. Lett. 2010, 12, 2206-2209 (DOI: 10.1021/ol100525r)

6. Boyd, D. R.; Sharma, N. D.; Sbircea, L. ; Murphy, D. ; Belhocine, T.; Malone, J. F.; James, S. L.; Allen C. C. R. ; Hamilton, J. T. G.; Azaarene cis-dihydrodiol-derived 2,2′-bipyridine ligands for asymmetric allylic oxidation and cyclopropanation, Chem. Commun., 2008, 5535-5537 (DOI: 10.1039/b814678k). See also

http://www.rsc.org/Publishing/Journals/cc/News/B814678K_Boyd_B812366G_James.asp

4. Details of the impact (indicative maximum 750 words)

Cardiome Pharma, a Canadian pharmaceutical company, recognised in 2003 that the team at QUB had the ability to deliver kilogram quantities, to GMP standard, of a chiral intermediate required for an alternative chemoenzymatic route to their new drug candidate RSD1235 for a clinical trial for the treatment of atrial fibrillation by intravenous injection. Atrial fibrillation, which is linked with strokes, is the most common form of irregular heartbeat, with an estimated nine million sufferers worldwide and established drugs for restoring normal heart rhythms are limited by either modest efficacy and/or side effects.

After investing more than CAN $1M prompted by research in two seminal publications by the Queen's group (Reference 1, J. Am. Chem. Soc., 1994, 116, 1147; Reference 2, J. Chem. Soc., Chem. Commun. 1995, 117 *in section 3*), during 2004 QUB was employed to deliver 5 kg of an enantiopure bioproduct and develop the synthetic chemistry used in the first synthetic steps toward RSD1235 (Reference 1 *in section 5*). Using this alternative improved chemoenzymatic route, Cardiome ultimately prepared 1 kg of homochiral RSD1235 to GMP standard required for clinical trials based on the material and routes Queen's provided (Reference 2 *in section 5*). As a result of the clinical trials, in 2009 Merck signed a licensing agreement with Cardiome worth up to 600 million dollars to help rapidly get the drug to market (Reference 3 *in section 5)*. RSD 1235 has completed clinical trials, is now marketed as Vernakalant and in September 2010 was approved for use in over ten European countries and other areas under the trade name Brinavess (Cardiome/Merck). Vernakalant is currently in phase 3 clinical trials for FDA approval for use in the USA and is currently being evaluated by the National Institute for Health and Clinical Excellence, NICE, as a prescription drug for NHS use in the UK (Reference 4 *in section 5*).

Secondly, Almac Sciences have over the past five years developed an increasing biocatalytic capability within their business facilitated by the research undertaken in Queen's led by Boyd and Stevenson. Many of the initial bioproducts marketed by Almac SelectAZyme, and new projects undertaken for external customers, were solely based on the biocatalytic pathways, enzymes, expertise and facilities used for the formation of polyoxygenated cyclohexanes from aromatics developed in Queen's which are utilized as precursors in synthetic routes to bioactive materials, such as the influenza drug Tamiflu (Reference 5 *in section 5*). The development of the routes for commericalisation was undertaken through a collaboration between the two groups of researchers (Reference 6 *in section 5*). This area is now growing into other markets significantly and the biocatalysis group in Almac now employ 30 staff including 15 PhD graduates from QUB trained in Boyd and Stevenson's groups. The biocatalysis group operates as a multi-million revenue provider for Almac providing solutions to customers through the application of enzymes.

Therefore, this biocatalysis research has led to a number of economic and health related impacts whose beneficiaries are primarily the industrial partners and the resultant economic benefits to the general public as well as the new development of treatments used in general practice worldwide.

5. Sources to corroborate the impact (indicative maximum of 10 references)

1: Letter of support from Cardiome:by Senior Director, Research (Chemistry)
"The route employing the *cis*-dihydrodiol as starting material was sufficiently promising that the process was optimized and demonstrated in the production of 1kg of cGMP vernakalant starting from 5kg of the *cis*-diol produced by Professor Boyd and his colleague Chris Allen".

2: The Cardiome request for chlorobenzene *cis*-dihydrodiol (5kg fermentation product) for 1kg GMP manufacture of RSD1235 by Raylo and a possible advantage of the new chemoenzymatic route based on an earlier (1 kg) delivery :-

"Not only has the new route of manufacture for RSD1235 been shown to be feasible on tens of gram scale by Raylo, but a significant improvement in yield from 40% to 65-70% for the last critical displacement step has been made by Raylo and Medichem."

3: Merck & Co. announces $600-million licensing agreement for Cardiome's vernakalant (www.firstwordpharma.com/node/359114)

4: NICE - Health Technology Appraisal for Vernakalant for the treatment of recent onset atrial fibrillation (http://guidance.nice.org.uk/TA/Wave26/7/DraftScope/pdf/English)

5: Almac.SelectAZyme brochure offering a new range of single enantiomer substituted benzene*cis*-diols.

6: Letter of support from Almac Sciences Ltd by Head of Biocatalysis & Isotope Chemistry.