1. **PUBLISHABLE SUMMARY**

**INTRODUCTION**

Human malignant pleural mesothelioma (MPM) is an aggressive thoracic cancer associated with previous asbestos exposure. Typically symptoms do not appear until 20-40 years after the original exposure, with life expectancy then 12-18 months. Given the great use of asbestos during the 20th century and this long latency period, it is no surprise that cases of mesothelioma continue to rise, with a peak not expected until 2020. At present, there are *ca*. 2450 deaths in the UK *per annum* from mesothelioma, and *ca*. 3,500 in the USA. There is no known cure for mesothelioma, and traditional chemotherapeutic cancer treatments, such as *cis*-platin or pemetrexed/*cis*-platin[1-4] have had little impact on the disease; radiotherapy and pleurectomy have only very limited impact on life expectancy.[5] Unfortunately there are no drugs in development specifically targeting this orphan disease. In particular, there are no reported good lead compounds (natural products) as a starting point for the drug discovery process. Until now.

First isolated from *Streptomyces* sp. AK-AB27 in 2008, JBIR-23 possesses a unique structural architecture, hitherto unobserved in *Streptomyces* extracts.[6] Furthermore, JBIR-23 is unique, in that it is the first natural product to be isolated that exhibits activity against human MPM (with IC50 values of 10 and 20 μM against the two main human mesothelioma cell lines).[6] These cell lines have demonstrated resistance to all known clinical anticancer agents. This is an amazing breakthrough: the isolation of the first natural product to be highly active against mesothelioma is crucial, providing a precious lead compound for investigation. An analogue, JBIR-24, **2**, has also been isolated from the same *Streptomyces* species, although showed slightly lower activity against mesothelioma cell lines.[6] Interestingly, the side chain of JBIR-23 is also observed in Cuevaenes A and B,[7] two metabolites isolated from *Streptomyces* sp. HKI0180 and it is not too difficult to imagine that these four compounds may have the same or similar biosynthetic origin.



In 2011, Takagi[8] reported that JBIR-23 exhibited significant antitumoral efficacy in MPM tumor xenografts in nude mice with no weight loss or any other obvious adverse effects. Furthermore, JBIR-23 not only prevented tumor growth but also led to a reduction in tumour volume. They concluded that, “these results suggest that JBIR-23 is a promising lead compound for use in MPM-specific therapeutic drugs without any side effects.”

**AIMS OF PROJECT**

This recent report made accessing synthetic routes to JBIR-23 and its analogues, for utilising in structure-activity studies even more timely and vital. Thus the overall aim of the project was to devise routes to JBIR-23 and also simplified versions of this compound, to permit biological evaluation and attempt to determine the origins of its biological activity.

**RESULTS**

The synthesis of analogues of JBIR-23 was performed by dissecting JBIR-23 into two independent portions: the synthesis of the fused three-ring system ABC, which was done by the fellow, and separately the long tri-ene side chain whose analogues were synthesized by a graduate student. The thinking behind this strategy was to prepare a number of compounds en route to the final target, all of which would be assessed for biological activity, in order to determine the minimal components of JBIR-23 required for biological activity (known as the pharmacophore).

The project was divided into two distinct parts: first the synthesis of compounds, and second their biological evaluation.

Within the synthetic studies, the simpler ABC system of Cuevaene A was first prepared. This enabled the Fellow to become familiar with the preparation of such compounds, before studying the more complex system related to JBIR-23. We cannot reveal the structures of all these compounds for reasons of confidentiality. A genuine sample of Cuevaene A was generously supplied to assist the studies by Prof. Richard Taylor from the University of York.

All the compounds prepared were tested against the MPM cell line MSTO by the Fellow, with the help of Dr Szlosarek’s research group at the Barts Cancer Institute. The occurrence of activity of some of the compounds gave us a clue to the key structural features and functional groups required for anti-mesothelioma activity. A colorimetric assay (MTS assay) was performed on the intermediates and analogues prepared, as well as on a sample of Cuevaene A. The side chain analogues, prepared by the graduate student, were also tested by the Fellow in the same assay.

Unlike JBIR-23, the natural product Cuevaene A showed no activity in the biological studies. This trend was continued in the side chain analogues, all of which showed poor and very low activity. Indeed, almost no difference was observed between Cuevaene A and any of the side chain analogues, indicating that this has little influence on the observed high activity of JBIR-23.

However, our synthetic strategy towards the total synthesis of the JBIR-23 molecule involved preparing, en route to the final target, a number of quinone compounds. A number of these quinines showed significant biological activity in the same assay described previous, and the activity was enhanced even further by preparing the corresponding epoxyquinones; thus even more closely resembling the BC system of JBIR-23. This gave us an idea of the epoxy group being important in the activity of JBIR-23. The structures are not included here for reasons of confidentiality. This lead us to prepare a further small library of simplified substituted epoxyquinones, the best of which showed an activity similar to that described for JBIR-23, with IC50 values around 10 – 15 μM against MSTO cell line. The decision to focus on this latter library of compounds meant that the total synthesis of JBIR-23 was not completed. These much simpler compounds are a great step forward in the simplification of the structure of JBIR-23 and represent an excellent starting point for further structure-activity optimisation studies.

**CONCLUSIONS AND FUTURE WORK**

Given the very limited laboratory-based mesothelioma research taking place in the world, the achievements of this project have opened a new field of study and provided preliminary structure-activity data around the structures of Cuevaene A and JBIR-23. These results have highlighted the key structural features of the natural product that we believe to be essential for biological activity and will form the foundation for further medicinal chemistry projects, both within the host group as well as indirectly by the Fellow whose knowledge acquired in this area will be transferable to her further career.

With this project, we have helped establish European competitiveness in this field, as there are no other chemistry laboratories within the EU preparing libraries of compounds to combat this neglected disease. Once the intellectual property associated with the project has been addressed fully, publications and wider dissemination of the results will follow.

**OUTREACH, IMPACT and PUBLIC DISSEMINATION**

Working within the confines of the confidential nature of the project, the Fellow has presented her work at the following external symposia:

i) **RSC Postgraduate Symposium of the Heterocyclic and Synthesis Group -** GlaxoSmithKline Medicines Research Centre, Stevenage (UK)**.** 18 September 2012

ii) **6th Postgraduate Symposium on Biological and Medicinal Chemistry -** Department of Chemistry – University of Cambridge (UK)**.** 14 December 2012

iii) **1st Symposium of the Society of Spanish Researchers in the United Kingdom-** Fundación Ramón Areces – Madrid (Spain). 1 April 2013

Furthermore, the scientist in charge (Dobbs) believes passionately in the public communication of science and also promotion of the dangers of asbestos and mesothelioma, partly through his own personal experience of the disease. He has spoken at 4 public communication events in association with mesothelioma, including Action Mesothelioma Day 2011 in Leeds (http://www.junehancockfund.org/2012/news-archive/action-mesothelioma-day/) and in Hampshire in 2012 (http://hasag.co.uk/).

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