Metabolic Study of New Psychoactive Substances

by

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A thesis submitted for the

Degree of Doctor of Philosophy (Science)

University of Technology, Sydney

2018

Certificate of original authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as part of the collaborative doctoral degree and/or fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Date: 10/01/2018

Acknowledgments

I would like to express my sincere gratitude to my supervisor Associate Professor Shanlin Fu for all your support and guidance. Your kind and cheerful manner was always encouraging and I would particularly like to thank you for the patience you had for me, which enabled me to finally reach the last stage of the challenging but rewarding journey.

To my co-supervisor, Dr Unnikrishnan Kuzhiumparambil, I would like to thank you for your various support in the lab, outside the lab and outside the university. You have been my mentor for research and beyond.

To the Swedish National Board of Forensic Medicine (Rättsmedicinalverket) and the Strategic Research Area Forensic Sciences (Strategiområdet Forensiska Vetenskaper) at Linköping University, particularly Dr Ariane Wohlfarth, Dr Robert Kronstrand, Dr Svante Vikingsson and Dr Henrik Green, I wish to thank you for the warm welcome in Sweden. The opportunity gave a whole new dimension to my project and enriched my cultural experience.

I would like to thank Advanced Chemistry Development (ACD/Labs) for providing MetaSense™ software as well as Richard Lee and Edward Milton for their help.

I thank you to all the academic and professional staff at UTS who supported me throughout my candidature. I wish to thank Jane Cameron and Zofia Winiarski for the support with the fungus study and allowing me the access to the incubation facility. Thank you to Dr Ronald Shimmon for providing the knowledge of synthetic techniques and support with the NMR and the laboratories. I also wish to thank Dr David Bishop and Dr Verena Taudte for the technical support with LC-MS instruments.

To Mahmoud El Safadi, thank you for the support with the synthesis. To Dr Susan Luong and Dr Anna Molnar, thank you for the tips for LC-MS use and PhD life. I also wish to thank Daniel Pasin, Morgan Philp and the forensic toxicology research group for providing valuable feedback at a number of occasions.

I would also like to thank Professor Claude Roux and Centre for Forensic Science for providing support, particularly with the attendance of conferences.

Finally, to all my friends and family, thank you for all your support, encouragement and fun times.

Publications

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For Chapter 2

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Abstract

The rapid and continuous emergence of new psychoactive substances has been a trend of the recreational drug market over the last decade. The sheer number of the new drugs has posed a challenge for the forensic and clinical laboratories to detect the consumption of the drugs in urine drug testing, especially when the metabolism of the drugs is unknown. This thesis aims to demonstrate that the fungus *Cunninghamella elegans* is a suitable model for metabolism studies of synthetic cannabinoids, as well as to provide new knowledge on metabolism of several new psychoactive substances to aid the forensic and clinical laboratories in detection of these drugs in urine testing.

Seven synthetic cannabinoids JWH-018, JWH-073, AM2201, 5F-PB-22, PB-22, XLR-11 and UR-144 were incubated with *C. elegans* for 72 h and the obtained metabolites were analysed by liquid chromatography–high resolution mass spectrometry (LC-HRMS). The cannabinoids underwent various biotransformations including, either alone or in combination, carboxylation, defluorination, dehydrogenation, demethylation, dihydrodiol formation, dihydroxylation, ester hydrolysis, hydroxylation, ketone formation, *N*-dealkylation, oxidative defluorination, oxidative defluorination to carboxylic acid, and trihydroxylation. Glucosidation and sulfation were the observed phase II biotransformations, although uncommon. The fungal metabolites were generally consistent with the human-relevant metabolites of these drugs reported in literature, except that glucuronidation is the common phase II human metabolic pathway instead of glucosidation and that the prevalence of the fungal metabolites was not always reflective of the human metabolite prevalence.

Large amounts of fungal metabolites of UR-144 were obtained in an upscaled experiment and analysed by nuclear magnetic resonance (NMR) spectroscopy after isolating several metabolites by preparative high performance liquid chromatography (HPLC). Ten metabolites were characterised including dihydroxy metabolites, carboxy and hydroxy metabolites, a hydroxy and ketone metabolite, and a carboxy and ketone metabolite. Use of these metabolites as reference standards for the UR-144 metabolites after human liver

microsomes (HLM) incubation indicated that a dihydroxy metabolite, carboxy and hydroxy metabolites, and a hydroxy and ketone metabolite generated by the fungus were also produced by HLM.

The metabolism of the synthetic cannabinoid AM1220 was investigated after incubation with HLM and the fungus using liquid chromatography–tandem mass spectrometry (LC-MS/MS) and LC-HRMS. AM1220 was found to be a high clearance drug and hydroxylation, demethylation and dihydrodiol formation were the major biotransformations.

The metabolism of acetylfentanyl, acrylfentanyl, 4-fluoro-isobutyrylfentanyl, and furanylfentanyl was studied in authentic human urine and human hepatocytes samples using LC-HRMS. *N*-dealkylation, hydroxylation, and hydroxylation and methoxylation were the major biotransformations for all but furanylfentanyl, which was mainly transformed by amide hydrolysis and dihydrodiol formation. It illustrates the need to examine the metabolism of individual drugs rather than predicting them based on the previous knowledge.

In conclusion, *C. elegans* has the ability to mimic human metabolism and, by allowing large production of metabolites, enables more comprehensive characterisation of metabolites by NMR analysis. Therefore, the fungus can be a useful complementary model for metabolism of synthetic cannabinoids.