UTS UNIVERSITY OF TECHNOLOGY SYDNEY

Cytotoxicity and Metabolic Study of New Psychoactive Substances

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Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy (Science)

under the supervision of Professor Shanlin Fu and Professor Paul Kenneth Witting

University of Technology Sydney
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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, *Huey Sze Leong* declare that this thesis, is submitted in fulfilment of the requirements for the award of *Doctor of Philosophy*, in the *School of Mathematical and Physical Sciences/ Faculty of Science* at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by the Australian Government Research Training Program.

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List of papers/publications

The following papers for Chapter 3 and Chapter 4 are reproduced in this thesis with the permission from the publishers (Creative Commons Attribution 4.0 International License, CC BY 4.0). Paper for chapter 5 has been submitted for peer-reviewed publications.

For Chapter 3

Leong, H.S.; Philp, M.; Simone, M.; Witting, P.K.; Fu, S. Synthetic Cathinones Induce Cell Death in Dopaminergic SH-SY5Y Cells via Stimulating Mitochondrial Dysfunction. *Int. J. Mol. Sci.* **2020**, *21*, 1370
https://doi.org/10.3390/ijms21041370

For Chapter 4

Huey Sze Leong, Shimpei Watanabe, Unnikrishnan Kuzhiumparambil, Ching Yee Fong, Hooi Yan Moy, Yi Ju Yao, Paul K Witting, Shanlin Fu. Monitoring metabolism of synthetic cannabinoid 4F-MDMB-BINACA via high-resolution mass spectrometry assessed in cultured hepatoma cell line, fungus, liver microsomes and confirmed using urine samples.
Forensic Toxicol. **2021**, *39,* 198–212
https://doi.org/10.1007/s11419-020-00562-7

For Chapter 5

Huey Sze Leong, Morgan Philp, Paul Kenneth Witting, Shanlin Fu. The Detox Factory: Toxicology Profile of New Psychoactive Substances.

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Data shown in panel F and G are mean ± SD obtained from four independent experiments. #### $p <$ 0.0001 vs 300 μM pentylone and 300 μM MDPV. Different to the control; * $p < 0.1$, ** $p < 0.01$ and **** $p < 0.0001$. RFU: Relative Fluorescence Unit

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Abstract

The unprecedented growth of new psychoactive substances (NPS) render identification a challenging issue to both the forensic and clinical laboratories. NPS readily available in myriad of unknown formulation, posing serious threat and acute harm for the users. At present, there is paucity of information on the potential potency, toxicity mechanisms, and toxicokinetic parameters associated with the use of these drugs. The present study aimed to investigate the neurotoxicity potency and cellular mechanism of NPS. Hepatoxicity potency potential, metabolic stability and subsequent metabolism pathway of specific NPS is also explored for better understanding of the toxicokinetics of these NPS.

The neurotoxicity potential and mechanism of synthetic cathinones (SCs) butylone, pentylone and 3,4-methylenedioxypyrovalerone (MDPV) was investigated using differentiated SH-SY5Y cell line. Viability assays and end-point measurements that include markers of oxidative stress, mitochondrial bioenergetics, intracellular calcium (Ca^{2+}) and cell death pathways were employed. All the three SCs displayed dose-dependent neurotoxicity with the following order of potency: butylone (least cytotoxic) < pentylone < MDPV (most cytotoxic). The activation of apoptotic cell death pathway implicated the orchestration of mitochondrial-mediated neurotoxicity mechanisms via oxidative stress, compromised bioenergetics balance and changes in Ca^{2+} homeostasis ($p < 0.0001$ vs. control).

The metabolism of synthetic cannabinoid (SCB), 4F-MDMB-BINACA was investigated using *in vitro* models: HepG2 liver cells, fungus *Cunninghamella elegans* (*C. elegans*) and pooled human liver microsomes (HLM). Tentative structure elucidation of the *in vitro* metabolites was performed using high-resolution mass spectrometry whilst twenty authentic human urine samples were retrospectively analysed using liquid chromatography-orbitrap mass spectrometry. A total of twenty-five *in vitro* metabolites and eight *in vivo* metabolites were tentatively identified. Ester hydrolysis and ester hydrolysis dehydrogenation 4F-MDMB-BINACA metabolites were recommended as urinary markers for 4F-MDMB-BINACA intake. *C. elegans* has the potential to be used

as a complementary model to predict and characterise human metabolites, as well as identifying possible drug toxicities for emerging SCBs.

The metabolic stability and hepatotoxicity potential of butylone, pentylone, MDPV and 4F-MDMB-BINACA were studied using HLM and HepG2 liver cells, respectively. Drug-treated HepG2 exhibited the following cytotoxicity potency: butylone (least cytotoxic) < pentylone < MDPV < 4F-MDMB-BINACA (most cytotoxic). For the metabolic stability study, NPS incubated in HLM were collected at various time points and subsequently analysed by liquid-chromatography tandem mass spectrometry. Calculated *in vitro* half-lives together with estimated intrinsic clearance values categorised butylone, pentylone and MPDV as low clearance drugs and 4F-MDMB-BINACA as high clearance drug.

Keywords: New psychoactive substances, cytotoxicity, metabolic profile, *in vitro*