

Metabolic Study of New Psychoactive Substances

by

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Certificate of original authorship

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Publications

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For Chapter 2

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2. Watanabe S, Kuzhiumparambil U, Winiarski Z, Fu S. Data on individual metabolites of synthetic cannabinoids JWH-018, JWH-073 and AM2201 by *Cunninghamella elegans*. *Data Brief*. 2016;7:332-40. doi:[10.1016/j.dib.2016.02.039](https://doi.org/10.1016/j.dib.2016.02.039).

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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.