Human phase I metabolism of the novel synthetic cannabinoid 5F-CUMYL-PEGACLONE

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Background

5F-CUMYL-PEGACLONE

Molecular Formula: $C_{25}H_{27}FN_2O$
Monoisotopic Mass: 390.2107
Binding Affinities:
K_i (hCB_1) 0.31 ± 0.07 nM
K_i (hCB_2) 1.56 ± 0.66 nM
(own unpublished data)

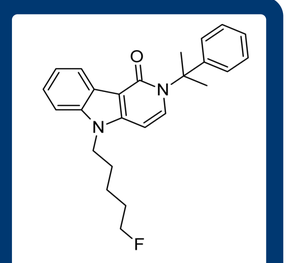

Fig.1: Structure 5F-CUMYL-PEGACLONE

- Street name: 5F-SGT-151
- 'Research chemical' purchased online Oct. 2017
  → Delivered Apr. 2018
- Notified Dec. 2017 by the EMCDDA
- 5-Fluoro analog of CUMYL-PEGACLONE [1]
  - 2nd emerged *γ*-carboline-1-one deriative

Objectives & Workflow

1 Analytical characterization of parent compound

Instrumentation
LC-MS/MS
UHPLC Nexera X2 (Shimadzu)
QTRAP® 5500 (Sciex)
LC-HR-MS
Elute (Bruker)
qToF-MS impact II™ (Bruker)
Chromatography
Kinetex® C18 (Phenomenex)

Prediction of phase I metabolites

Pooled human liver microsome (pHLM) assay
No reference standards for metabolites available
→ HLM incubation with parent compound
→ Generation of phase I metabolites

2 *In vitro* metabolite characterization

Implementation in LC-MS/MS routine screening

3 *In vivo* metabolite identification

Sample preparation urine
→ 0.5 mL + β-glucuronidase (1 h, 45 °C)
→ Liquid/liquid-extraction (ACN / $NH_4^+HCOO^-$)

Method update with most suitable metabolites

Authentic case samples
→ *In vivo* confirmation with authentic human urine samples
→ Qualitative metabolite ranking
→ Selectivity & sensitivity testing

4 *In vivo* biomarker evaluation

Results

Collision induced dissociation (CID) (Fig 2.) of the parent compound led to the main fragment ion ⓐ, which produces fragment ⓑ by the loss of HF. Elimination of the cumyl moiety leads to the dimethylbenzyl ion ⓒ and the tropylium ion ⓓ. A characteristic fragmentation pathway for *γ*-carbolinone based synthetic cannabinoids is the formation of the three core fragment ions ⓔ, ⓕ and ⓖ. [1]

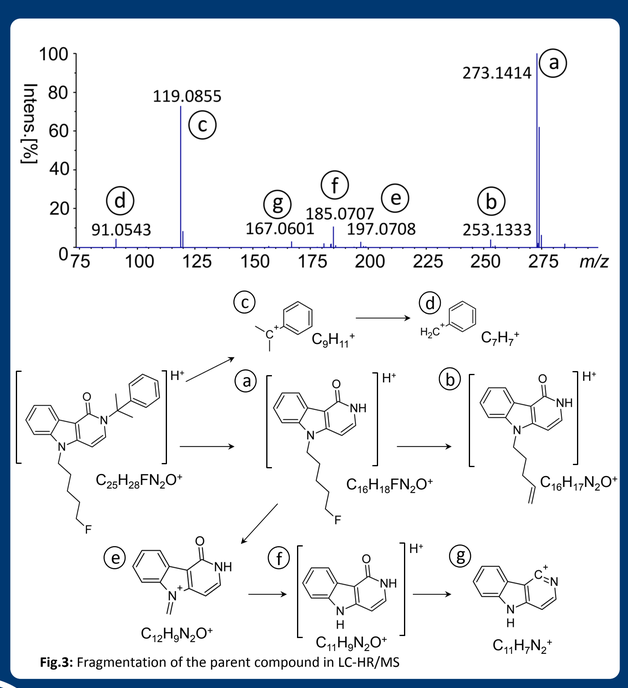

Fig.3: Fragmentation of the parent compound in LC-HR/MS

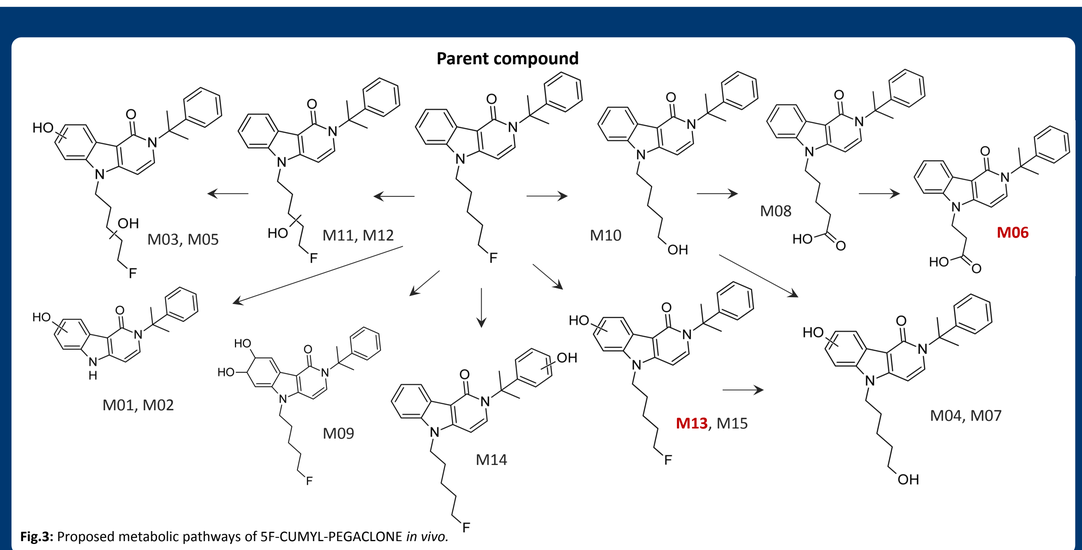

Fig.3: Proposed metabolic pathways of 5F-CUMYL-PEGACLONE *in vivo*.

In total, 30 *in vitro* phase I metabolites were generated in the pHLM assay and characterized by LC-qToF-MS. The most abundant metabolites M10 and M12 were integrated in a LC-MS/MS routine screening method. Subsequently, 13 authentic urine samples from forensic casework were found positive for M10 and M12. These samples were used to detect possible metabolites not generated in the pHLM assay and to evaluate the most suitable *in vivo* phase I metabolites for a detection of 5F-CUMYL-PEGACLONE use in urine samples. 15 *in vivo* metabolites were identified in the investigated set of urine samples. The metabolic reactions *in vivo* (see Fig 3.) included hydroxylation, formation of a dihydrodiol, hydrolytic defluoration, *N*-dealkylation, oxidation to the pentanoic acid metabolite and side chain degradation to a propionic acid metabolite, which has already been described for other SCs with a 5-fluoropentyl side chain. [2]

In human phase I metabolism, the 5-fluoropentyl chain and the *γ*-carbolinone core were the preferred moieties for biotransformations. The parent compound was not detected in any of the authentic urine samples. In total, 12 out of 15 *in vivo* phase I metabolites could be confirmed by corresponding signals in the pHLM assay. M06 was the most abundant metabolite in each of the analyzed urine samples but could not be detected *in vitro*.

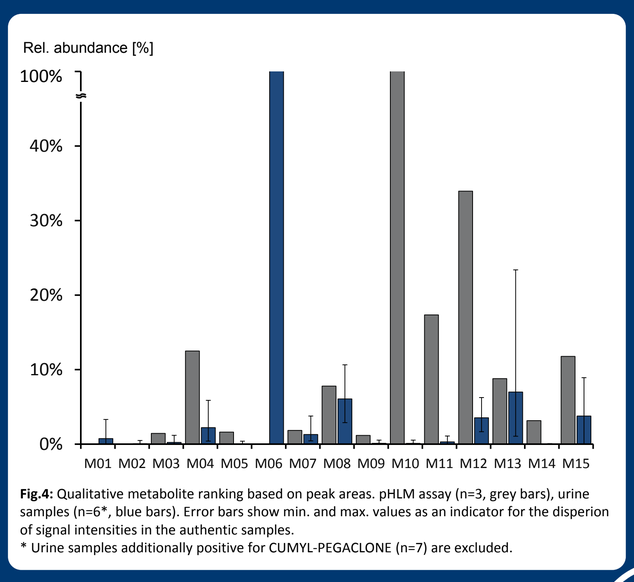

Fig.4: Qualitative metabolite ranking based on peak areas. pHLM assay (n=3, grey bars), urine samples (n=6*, blue bars). Error bars show min. and max. values as an indicator for the disperion of signal intensities in the authentic samples.
* Urine samples additionally positive for CUMYL-PEGACLONE (n=7) are excluded.

Discussion

Comparing the results to the human phase I metabolism data of the non fluorinated analog CUMYL-PEGACLONE [3], identical metabolites may occur for both compounds. Analyzing six urine samples positive for CUMYL-PEGACLONE only, six identical metabolites (M01, M02, M04, M06, M08, M10) were detected (see Fig. 5). Thus, the main *in vivo* metabolite M06 can be used in screening methods when maximum sensitivity is needed but not to differentiate between the uptake of the two analogs. Additional detection of M13, monohydroxylated at the core system with an unaltered 5-fluoropentyl chain, will facilitate a selective detection of 5F-CUMYL-PEGACLONE uptake (see Fig. 6).

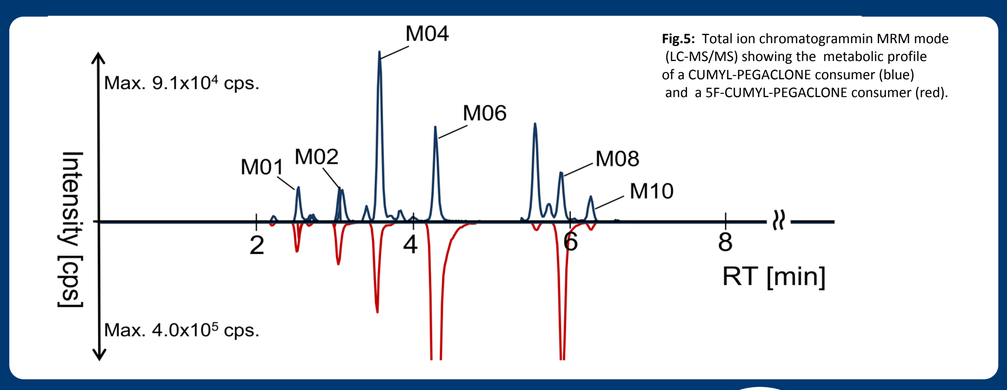

Fig.5: Total ion chromatogrammin MRM mode (LC-MS/MS) showing the metabolic profile of a CUMYL-PEGACLONE consumer (blue) and a 5F-CUMYL-PEGACLONE consumer (red).

Conclusions

- ✓ 5F-CUMYL-PEGACLONE is subject to extensive metabolism in humans.
- ✓ A characteristic CID pathway of *γ*-carbolinone derivatives was confirmed.
- ✓ M06 is a sensitive marker metabolite for 5F-CUMYL-PEGACLONE uptake but can also be formed when CUMYL-PEGACLONE was the drug of abuse.
- ✓ M13 facilitates a selective detection of 5F-CUMYL-PEGACLONE uptake.
- ✗ The degradation pathway of the 5-fluoropentyl chain to a propionic acid metabolite remains subject to further metabolism studies.

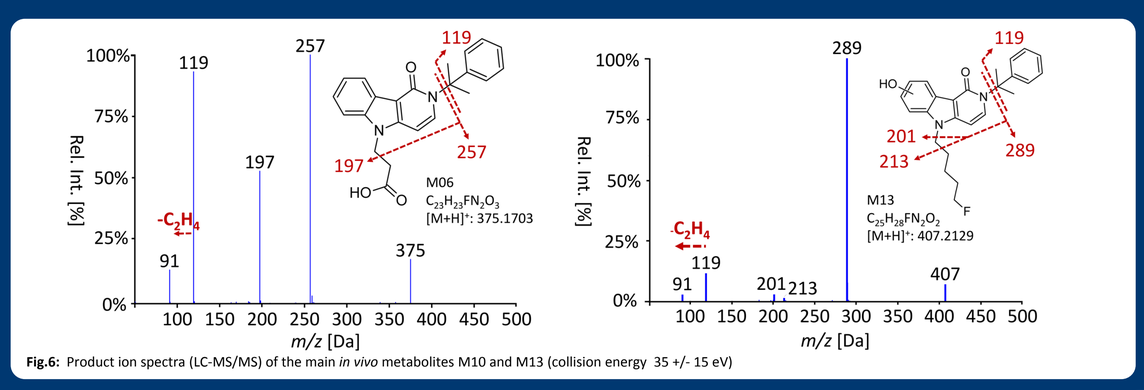

Fig.6: Product ion spectra (LC-MS/MS) of the main *in vivo* metabolites M10 and M13 (collision energy 35 +/- 15 eV)

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Literature

[1] Angerer *et. al.* Structural characterization and pharmacological evaluation of the new synthetic cannabinoid 'CUMYL-PEGACLONE'. Drug Test. Anal. 2018 Mar;10(3):597-603. doi: 10.1002/dta.2237.

[2] Mogler *et. al.* Detection of the recently emerged synthetic cannabinoid 5F-MDMB-PICA in 'legal high' products and human urine samples. Drug Test. Anal. 2018 Jan;10(1):196-205. doi: 10.1002/dta.2201.

[3] Mogler *et. al.* Phase I metabolism of the recently emerged synthetic cannabinoid CUMYL-PEGACLONE and detection in human urine samples. Drug Test. Anal. 2018 May;10(5):886-891. doi: 10.1002/dta.2352.

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