Phase I metabolism of the synthetic cannabinoid CUMYL-PEGACLONE

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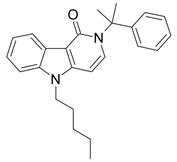
Background



IUPAC:
5-pentyl-2-(2-phenylpropan-2-yl)-2,5-dihydro-1*H*-pyrido[4,3-*b*]indol-1-one

Semi-systematic name:
2-cumyl-5-pentyl gamma-carboline-1-one

Fig. 1: Chemical structure of CUMYL-PEGACLONE.

Synthetic cannabinoid receptor agonists (SCRAs) are a structurally diverse class of new psychoactive substances (NPSs). Most SCRAs are based on indole or indazole core structures. **CUMYL-PEGACLONE** emerged on the German drug market in December 2016. In contrast to SCRAs like CUMYL-PICA published by Bowden and Williamson [1], the linker group, connecting a cumyl substituent to the core structure, is included into a **tricyclic *γ*-carboline**. The substance shows high binding affinity for the human cannabinoid receptors.[2]

Objectives

- Identification of metabolic pathways
- Evaluation of biomarkers in urine
- Application to authentic urine samples

Analytical Workflow

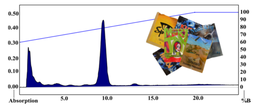


Fig. 2: Flash-chromatogram of the extract from a 'herbal mixture'.

No reference standards of CUMYL-PEGACLONE and its metabolites were commercially available.
The compound was isolated from a 'herbal mixture' by semi-preparative **flash chromatography.**

Pooled human liver microsomes **(pHLM)** were incubated with the isolated substance to generate ***in vitro* phase I metabolites**, and to obtain reference spectra by LC-MS/MS and LC-qTOF-MS. The ion transitions of the tentative *in vitro* main metabolites were integrated into an existing LC-MS/MS screening method.

Sample preparation pHLM
Incubation 1 h at 37 °C
Extraction with ACN

Sample preparation urine
0.5 mL urine + β-glucuronidase (1 h, 45 °C)
Extraction (ACN / 10 M $NH_4^+HCOO^-$)

LC-Instrumentation
UHPLC Nexera X2 (Shimadzu)
Kinetex® C18 column
(100 mm × 2.1 mm, 100 Å, 2.6 µm)

MS-Instrumentation
QTRAP® 5500 (Sciex)
qToF-MS impact II™ (Bruker)

Subsequently urine samples were screened resulting in 135 positive samples for CUMYL-PEGACLONE until August 2017. **30 urine samples** were used **to identify the main *in vivo* phase I metabolites** as reliable biomarkers for drug uptake after enzymatic cleavage of conjugates.

Results & Discussion

27 *in vitro* phase I metabolites were detected in the pHLM samples incubated with CUMYL-PEGACLONE. The metabolites were characterized in LC-MS/MS multiple reaction monitoring (MRM) mode and by enhanced product ion (EPI) scans. Findings were supported by HR-MS. Seven biotransformation types could be assigned to the metabolites by comparing the EPI scan of a metabolite to the EPI scan of the parent compound.

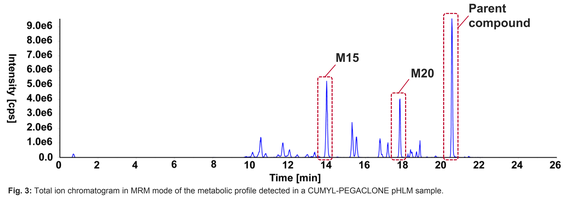


Fig. 3: Total ion chromatogram in MRM mode of the metabolic profile detected in a CUMYL-PEGACLONE pHLM sample.

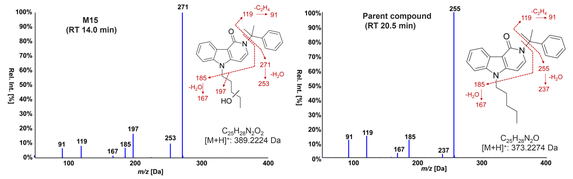


Fig. 4: EPI spectra of the main *in vitro* phase I metabolite M15 and the parent compound recorded from a pHLM sample of CUMYL-PEGACLONE.

M15 was the most abundant *in vitro* phase I metabolite. The EPI spectrum of *m/z* 389 (RT 14.0 min) with fragment ions *m/z* 271 and 197 indicate a **mono-hydroxylation at the pentyl side chain** (see Fig. 4). **M20** (*in vitro* rank position 2) is mono-hydroxylated **at the *γ*-carboline core** with the fragment ions *m/z* 271 and 201 in the EPI spectrum of *m/z* 389 (RT 17.8 min). The respective ion transitions were initially implemented in a routine screening method.

22 *in vivo* phase I metabolites were detected in the authentic urine samples (n=30). The metabolic reactions included mono- and di-hydroxylation, *N*-dealkylation, dehydrogenation, and the formation of a carbonyl function at the pentyl moiety in combination with mono-hydroxylation.

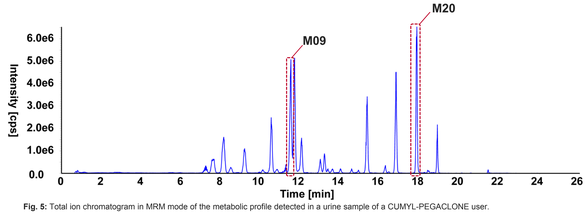


Fig. 5: Total ion chromatogram in MRM mode of the metabolic profile detected in a urine sample of a CUMYL-PEGACLONE user.

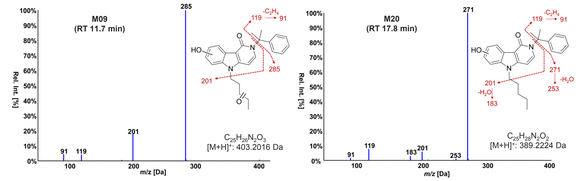


Fig. 6: EPI spectra of the main *in vivo* phase I metabolites M09 and M20 recorded from a urine sample of a CUMYL-PEGACLONE user.

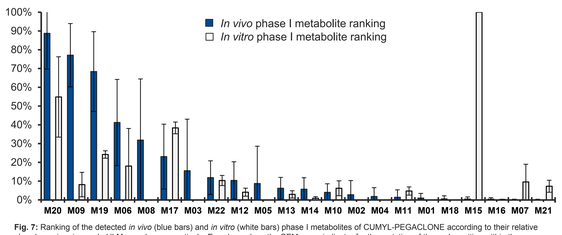


Fig. 7: Ranking of the detected *in vivo* (blue bars) and *in vitro* (white bars) phase I metabolites of CUMYL-PEGACLONE according to their relative abundance in urine and pHLM samples, respectively. Error bars show the SEM as an indicator for the variation of the rank position within the investigated collective.

M20 was the most abundant metabolite in the investigated set of urine samples. **M09** (*m/z* 403; *in vivo* rank position 2) is a product of mono-hydroxylation at the *γ*-carboline core in combination with a carbonyl function located at the pentyl side chain (see Fig. 6). The main *in vitro* metabolite M15 was detected *in vivo* but was a minor metabolite in human phase I metabolism. The **parent compound was not detected** in any of the urine samples.

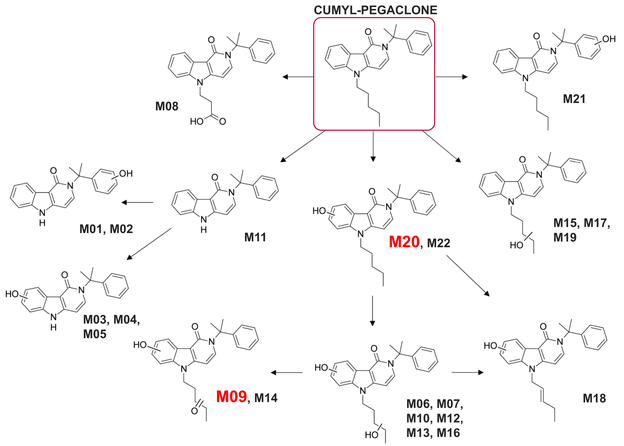


Fig. 8: Postulated *in vivo* phase I metabolic pathways of CUMYL-PEGACLONE. The main *in vivo* phase I metabolites are highlighted in red.

Conclusions

CUMYL-PEGACLONE is subject to extensive metabolism in humans. In human phase I metabolism, metabolic reactions occurred mainly at the pentyl moiety and the *γ*-carboline core. The metabolites **M20 and M09** were evaluated **as reliable biomarkers** to prove CUMYL-PEGACLONE consumption in urine samples. *In vitro* experiments using pHLMs showed to be a suitable approach to identify main phase I metabolites and generate reference spectra of phase I metabolites when reference standards of new SCRAs and their relevant main metabolites are missing. From the date of the emergence in December 2016 until August 2017, the prevalence of CUMYL-PEGACLONE in SCRA positive urine samples tested was 28%. The wide distribution of a SCRA with high binding affinity might pose risks to consumers like overdosing and experiencing adverse effects. It seems likely that further *γ*-carboline derivatives will occur as drugs of abuse in the future.

Aknowledgements

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[1] M.J. Bowden, J.P.B. Williamson. Cannabinoid compounds. WO2014167530 A1, 2014.

[2] Angerer et. al. Structural characterization and pharmacological evaluation of the new synthetic cannabinoid 'CUMYL-PEGACLONE'. *Drug Test Anal*, 2017. http://dx.doi.org/10.1002/dta.2237