Phase I metabolism of the carbazole derivatives EG-018 and MDMB-CHMCZCA – A new class of synthetic cannabinoids circumventing the 'NpSG'

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Current legal status

The German 'Act to control the distribution of new psychoactive substances' (NpSG) became effective on 26th of November 2016. According to the law, synthetic cannabinoids with indole, indazole or benzimidazole core structures are controlled.[1] EG-018, EG-2201 (5-fluoro-pentyl analog of EG-018) and MDMB-CHMCZCA are not covered by the 'NpSG' due to their carbazole core structures.

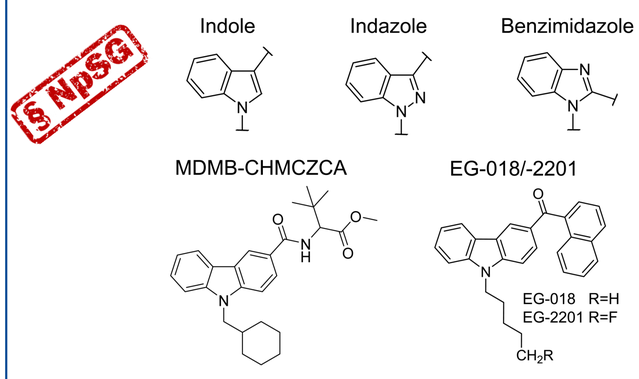

Fig. 1: Controlled core structures by the 'NpSG' and chemical structures of known carbazole derivatives.

Aims of the study

Phase I metabolism studies were conducted to identify biomarkers for the detection of EG-018 and MDMB-CHMCZCA metabolites in urine samples. Reference spectra of *in vitro* phase I metabolites generated by pooled human liver microsome assays were used as a positive control as reference standards of metabolites were not commercially available.

Sample preparation

***In vitro* microsomal phase I metabolism**

- Pooled human liver microsomes[2]
- Incubation 1 h at 37 °C with parent substance
- Extraction with ACN

***In vivo* phase I metabolism**

23 urine samples from forensic casework: EG-018 (n=8), MDMB-CHMCZCA (n=15)

- Incubation with β-glucuronidase (1 h, 45 °C)
- Extraction with ACN / 10 M $NH_4^+HCOO^-$

Analytical workflow

In vitro

Identification of main metabolites

In vivo

Implementation in routine urine screening

Phase I biomarker evaluation → **Optimization of the screening method**

Instrumentation:

LC-MS/MS QTRAP® 5500 (Sciex)
- Enhanced product ion scan (EPI)
- Precursor scan (Prec)
- Multiple reaction monitoring (MRM)

LC-QToF-MS impact II® (Bruker)
- Auto MS/MS
- Full scan & Broad band collision induced dissociation (bbCID) mode

Results for EG-018

In total, 25 metabolites were generated in pHLM samples incubated with EG-018.
Comparing the microsomal metabolic profiles of EG-018 and EG-2201, one identical metabolite (M*01) was detected, formed by mono-hydroxylation and hydrolytic defluorination, respectively.

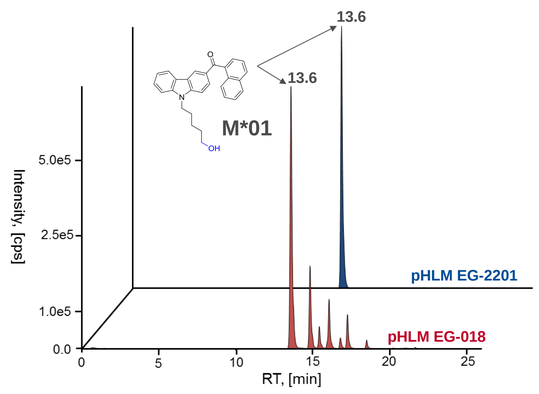

Fig. 2: Extracted ion chromatograms in positive MRM-Mode at *m/z* 408 → 155 (mono-hydroxylation) of pHLM samples of EG-018 and EG-2201.

From a total of 13 *in vivo* phase I metabolites of EG-018, detected in the urine samples, the ten most abundant metabolites were referred to four different biotransformation steps and confirmed by corresponding signals in the pHLM assay. M*04 a product of N-Desalkylation and hydroxylation was the most abundant *in vivo* metabolite. M*01, the 5-OH-pentyl metabolite, was also detectable in each urine sample but with a higher variation of its relative intensity than M04 among the investigated collective.

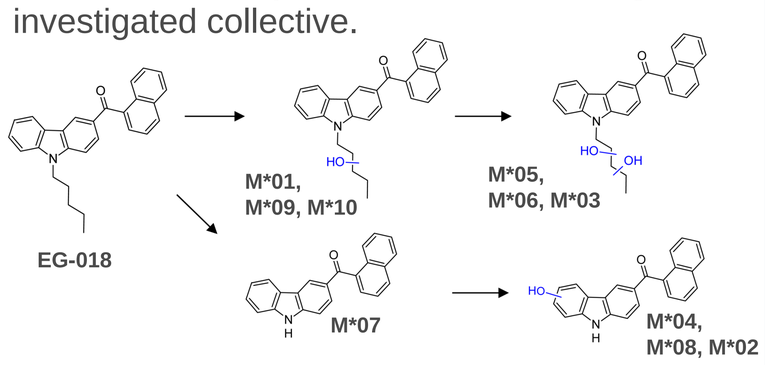

Fig. 3: Postulated main phase I metabolic pathways of EG-018 *in vivo*.

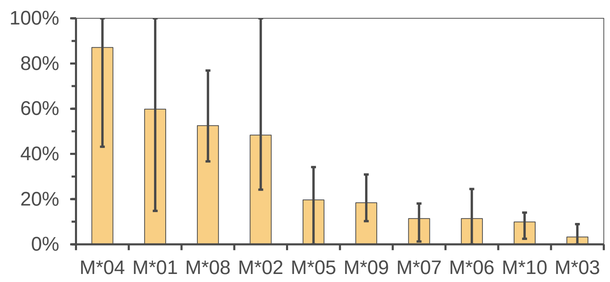

Fig. 4: Ranking of 10 detected *in vivo* phase I metabolites of EG-018 according to their relative abundance in 8 authentic urine samples. Error bars show the lowest and highest relative abundances within the investigated collective.

Results for MDMB-CHMCZCA

For MDMB-CHMCZCA, 29 metabolites were identified in the human urine samples and confirmed *in vitro* by corresponding signals in the pHLM assays. The ten most abundant metabolites were referred to five different metabolization steps and evaluated as reliable urinary biomarkers.

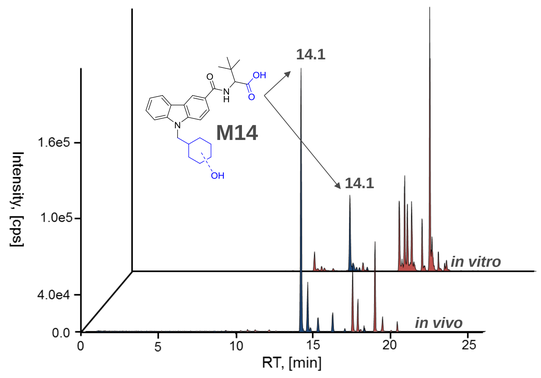

Fig. 5: Extracted ion chromatograms in MRM-Mode at *m/z* 451 → 306 (mono-hydroxylation in red) and m/z 437 → 306 (mono-hydroxylation + ester hydrolysis in blue) of a urine sample compared to a pHLM sample.

M14, formed by terminal ester hydrolysis and mono-hydroxylation of the cyclohexyl methyl moiety, was the most abundant metabolite among the MDMB-CHMCZCA positive urine samples. M07, mono-hydroxylated but with an intact methyl ester function, can be used to differentiate between the uptake of other chemically similar *tert*-leucine derivatives.

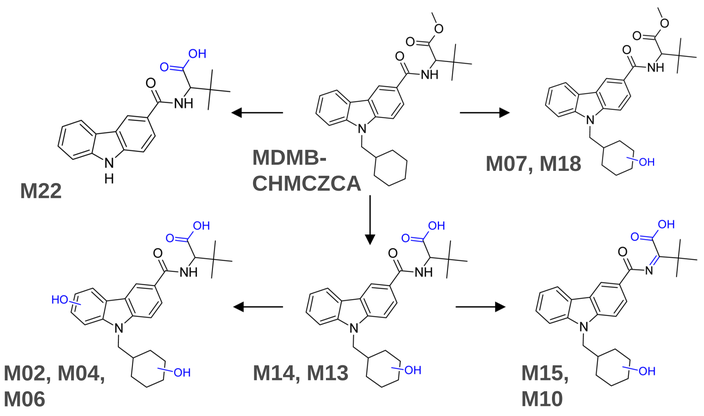

Fig. 6: Postulated main phase I metabolic pathways of MDMB-CHMCZCA *in vivo*.

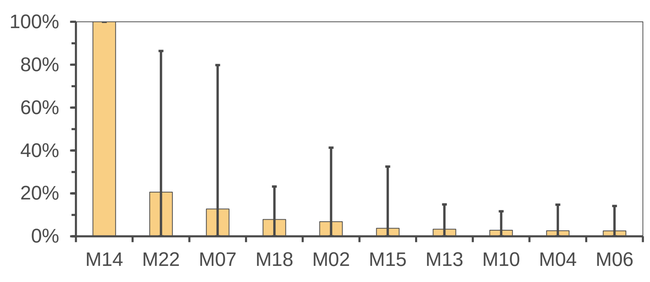

Fig. 7: Ranking of 10 detected *in vivo* phase I metabolites of MDMB-CHMCZCA according to their relative abundance in 15 authentic urine samples. Error bars show the lowest and highest relative abundances within the investigated collective.

Conclusions

M*04, M*01 are suggested as suitable urinary targets to prove EG-018 consumption in urine. For MDMB-CHMCZCA, the metabolites M14 and M07 are characteristic metabolites for urine analysis. Current online monitoring of 'legal high' products (see poster 32) indicates that carbazole derivatives, mainly MDMB-CHMCZCA, are sold via the Internet as legal alternatives to the recently banned SCs scheduled under the 'NpSG'. Therefore, we recommend to update LC-MS/MS screening methods with the respective metabolites.

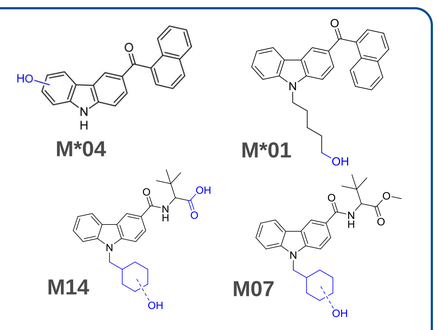

Fig. 8: Chemical structures of suggested urinary biomarkers, M*04 and M*01 (EG-018), M14 and M07 (MDMB-CHMCZCA).

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Literature

[1] Neue-psychoaktive-Stoffe-Gesetz (NpSG), Gesetz zur Bekämpfung der Verbreitung neuer psychoaktiver Stoffe.
[2] Mammalian Liver Microsomes, Guidelines for Use TF000017 Rev 1.0 (BD Biosciences)

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