In vitro metabolic profiling of the synthetic cannabinoid receptor agonist CUMYL-THPINACA using high resolution mass spectrometry

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Background
- Synthetic cannabinoid receptor agonists (SC) remain popular drugs of abuse.1
- Currently, SC-adulterated CBD-hemp is sold as drug-hemp.
- Effects comparable to THC, but generally far more potent.
- Severe health risks for consumers due to high potency, reflected in several (lethal) overdose cases.2
- Forensic and clinical analyses: Knowledge on the in vivo metabolism of SC are a prerequisite for detection of an ingestion as metabolites are the main targets in urine screenings.3
- Information on the metabolizing isoenzymes helps predicting metabolic drug-drug interactions.4-5

Material & Methods

In vitro metabolism:
- Pooled human liver microsomes (pHLM)
- Recombinant Cytochrome P450 liver enzymes (rCYP: CYP3A4, CYP2C9, CYP2E1, CYP3A5, CYP2C8, CYP2B6, CYP2A6, CYP1A2, CYP2D6, CYP2C19, CYP3A5, CYP2B6 )

Sample preparation (Figure 1):
1. Incubation with enzymatic model system for 0.5; 1; 1.5; and 2 h.
2. Solid phase extraction (SPE, Strata Phenyl cartridge) of incubation solution

Results & Discussion

Discovery: 28 Metabolites of CUMYL-THPINACA
- CUMYL-THPINACA was extensively hydroxylated, resulting in various mono-, di-, and tri-hydroxylated metabolites (Figure 2 and Table 1)
- Additional metabolites were formed by oxidation and subsequent dehydration at the oxacyclohexane moiety.
- Due to in-source dehydration being observed for M21* and M22*, further investigation is required of corresponding di- and tri-hydroxylated + dehydrated metabolites that are co-eluting with the respective di- and tri-hydroxylated metabolites.
- CUMYL-THPINACA was metabolized by various CYP-isofroms, mainly: CYP3A4, CYP3A5, CYP2C8, CYP2D6, and 2C19. Relevant metabolic drug-drug interactions are, therefore, not expected.

Table 1: Summary of all detected metabolites

<table>
<thead>
<tr>
<th>Transformation</th>
<th>Formula</th>
<th>[M+H]+</th>
<th>Mass change</th>
<th>Number of metabolites</th>
<th>Label</th>
<th>Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>monohydroxylation</td>
<td>C23H27N3O2</td>
<td>378.2176</td>
<td>+O</td>
<td>3.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dihydroxylation</td>
<td>C23H27N3O4</td>
<td>410.2074</td>
<td>+2 x O</td>
<td>5</td>
<td>M14, M19, M23</td>
<td>1.54, 1.81, 2.39</td>
</tr>
<tr>
<td>dihydroxylation + dehydration</td>
<td>C23H27N3O5</td>
<td>426.2023</td>
<td>+3 x O (47.9847 amu)</td>
<td>7</td>
<td>M2a-b, M11a-b, M4, M6, M8, M10, M12, M13</td>
<td>0.9-1.0, 1.08, 1.19, 1.23, 1.27, 1.42</td>
</tr>
<tr>
<td>trihydroxylation + dehydration</td>
<td>C23H27N3O6</td>
<td>408.1918</td>
<td>+3 x O (29.9741 amu)</td>
<td>6</td>
<td>M3, M5, M7, M15, M17, M20</td>
<td>1.08, 1.16, 1.20, 1.82, 1.88</td>
</tr>
<tr>
<td>CUMYL-THPINACA (parent)</td>
<td>C23H27N3O2</td>
<td>378.2176</td>
<td></td>
<td></td>
<td></td>
<td>3.07</td>
</tr>
</tbody>
</table>

Conclusion & Outlook
- CUMYL-THPINACA is extensively metabolized, as observed for other SCs. Therefore, metabolites need to be considered when screening urine samples.
- To rule out in-source dehydration, further investigation, and potentially sample derivatization, should be conducted.
- No drug-drug interactions are expected - as several CYP isofroms contribute to the metabolism of CUMYL-THPINACA.
- In vitro models are not able to fully mimic the in vivo situation: in vivo analysis of authentic urine samples is required to elucidate the most relevant metabolites.
- Further investigations will include analysis of authentic urine samples (where available), activity studies of metabolites, and occurrence of in-source dehydration.

References: