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# Synthetic cannabinoid BB-22 (QUCHIC): Human hepatocytes metabolism with liquid chromatography-high resolution mass spectrometry detection

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ABSTRACT

Clandestine laboratories continue producing new synthetic cannabinoids that mimic and magnify natural cannabinoids effects to circumvent drug scheduling legislation. New synthetic cannabinoids are highly potent and responsible for many acute intoxications and deaths. Characterization of metabolic pathways is critical to identify metabolite markers whose detection can prove intake. BB-22 is a new potent synthetic cannabinoid whose toxicological and metabolic properties are currently unavailable. Analytical methods require constant updating and are challenging due to extensive synthetic cannabinoid metabolism and low marker concentrations. A single non-specific BB-22 metabolite was previously identified in incubations with human liver microsomes (BB-22 3-carboxyindole). Clear characterization of BB-22's metabolism is required to help toxicologists document BB-22 consumption in clinical and forensic cases. We incubated 10 μmol/L BB-22 with cryopreserved human hepatocytes for 3 h. Samples were analyzed by liquid chromatography on a biphenyl column and high resolution mass spectrometry. Results were processed with data mining software, identifying ten metabolites. Loss of the quinolinyl side-chain via ester hydrolysis was the main biotransformation. All other metabolites were produced by further indole or cyclohexylmethyl hydroxylation or glucuronidation. We recommend BB-22 3-carboxyindole and two BB-22 3-carboxyindole-hydroxycyclohexylmethyl isomers as metabolite targets for documenting BB-22 intake. Hydrolysis of biological samples before analysis is strongly suggested to improve detection of phase I metabolites. BB-22 3-carboxyindole is not specific for BB-22 intake, as it was previously detected as a minor MDMB-CHMICA and ADB-CHMICA metabolite. Consumption of these two synthetic cannabinoids should be ruled out to confirm BB-22 intake.

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## 1. Introduction

BB-22 (8-Quinolinyl 1-(cyclohexylmethyl)-1H-indole-3-carboxylate), also known as QUCHIC, is a synthetic cannabinoid (SC), a manufactured substance produced to mimic and magnify the effects of natural cannabinoids. BB-22 was first identified in 2013 in illegal products, in Japan [1], and in 2016 in Europe [2]. BB-22 is readily available worldwide via specialized websites where it is sold as a "research chemical", but, unfortunately, there are no published data on BB-22 consumption.

BB-22 is composed of an indole core substituted by a cyclohexylmethyl tail at the nitrogen and a quinolinyl side chain via a carboxylate linker at $C_3$ (Fig. 1). Its structure is similar to MDMB-CHMICA, ADB-CHMINACA, PB-22, and 5F-PB-22, four SC

*Abbreviations:* SC, synthetic cannabinoid; $CB_1$, central cannabinoid receptors; $CB_2$, peripheral cannabinoid receptors; HRMS, high resolution mass spectrometry; FullMS, full-scan mass spectrometry; $ddMS^2$, data-dependent tandem mass spectrometry; AGC, automatic gain control; IT, injection time; AIF, all-ion fragmentation; RT, retention time.

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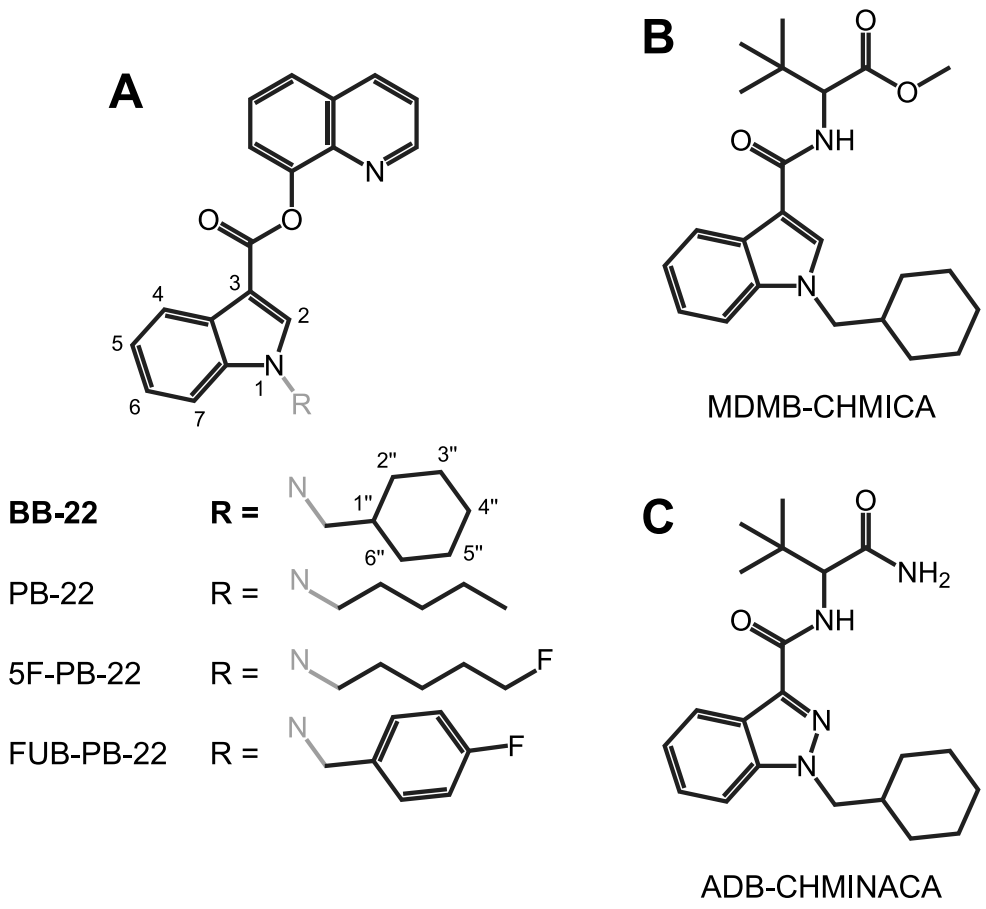


**Fig. 1.** Chemical structures of BB-22 (A) and structural analogs PB-22 (A), 5F-PB-22 (A), FUB-PB-22 (A), MDMB-CHMICA (B), and ADB-CHMINACA (C). Atoms were numbered for metabolite identification.

involved in multiple fatalities [3–5] (Fig. 1). BB-22 is much more potent than $\Delta^9$-tetrahydrocannabinol [6], and is a full agonist of central ($CB_1$) and peripheral ($CB_2$) cannabinoid receptors with affinities of $K_i$ = 0.11–0.217 nM and $K_i$ = 0.338 nM, respectively [7,8]. BB-22 binding affinity for the $CB_1$ receptor is lower than that of ADB-CHMINACA [9]. Half maximal effective concentration for $CB_1$ activation is $EC_{50}$ = 2.9 nM [7], similar to ADB-CHMINACA, PB-22, 5F-PB-22, and MDMB-CHMICA $EC_{50}$ [9–11]. Despite BB-22's potency, few intoxications were reported, and always with other SC [12–14]. BB-22, AB-CHMICA, and 5F-AKB-48 were detected in the plasma of a patient who had abdominal pains, reduced Glasgow score, seizures, tachycardia, hypertension, agitation, confusion, and acidosis [14]. These clinical symptoms are not specific for BB-22 intake and cannot be used to prove consumption.

Analytical identification is also challenging as BB-22 blood concentrations are low due to its high potency [7,8]. BB-22 plasma concentrations 5.5 and 8.3 h after intake were 97 and 94 ng/L, respectively, in a recreational consumption case. Four other SC were also found in similar concentrations [12]. In another case, a patient was admitted to the Emergency Department with 60 ng/L serum BB-22, with two other SC [13]. BB-22 is expected to be extensively metabolized, considering metabolism of structural analogs, such as PB-22, 5F-PB-22, and FUB-PB-22 [15,16] (Fig. 1). Urine metabolites detection documents evidence of SC intake and extends the detection window [17]. However, BB-22 metabolism is not fully characterized. In a recent study on MDMB-CHMICA metabolism, a single BB-22 metabolite (BB-22 3-carboxyindole) was identified following BB-22 incubation with human liver microsomes (HLM) [18]. Interestingly, the same metabolite was identified in MDMB-CHMICA smoke condensate and in vitro and in vivo MDMB-CHMICA metabolism [18]. MDMB-CHMICA is a scheduled SC in many countries [19] because of acute toxicity cases and deaths [3,20]. Clear distinction of BB-22 intake is critical and BB-22 metabolism pathways need to be thoroughly examined.

We investigated BB-22 metabolism after incubation with human hepatocytes and liquid chromatography-high resolution tandem mass spectrometry (LC-HRMS/MS), as previously recommended [21]. Although incubation with HLM is the most common approach for characterizing drug metabolism, human hepatocytes in vitro metabolism proved more suitable for SC metabolite profiling [16,22,23]. Results were compared to the only commercially available standard of a theoretical BB-22 metabolite to confirm our metabolite identification.

## 2. Materials and methods

### 2.1. Chemicals and reagents

BB-22 (8-Quinolinyl 1-(cyclohexylmethyl)-1H-indole-3-carboxylate) and BB-22 3-carboxyindole (1-(cyclohexylmethyl)-1H-indole-3-carboxylic acid) were purchased from Cayman Chemical (Ann Arbor, MI, USA) and dissolved in methanol to 1 g/L. Diclofenac standard was purchased from Toronto Research Chemicals (Toronto, Canada). LC–MS grade water, methanol, and formic acid (Optima™ LC/MS) were acquired from Fisher Scientific (Fair Lawn, NJ, USA), and LC–MS grade acetonitrile from Sigma-Aldrich® (St. Louis, MT, USA). Ten-donor-pooled cryopreserved human hepatocytes were obtained from BioreclamationIVT (Baltimore, MD, USA).