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Tentative identification of phase I metabolites of HU-210, a classical synthetic cannabinoid, by LC–MS/MS

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ABSTRACT

(6a*R*,10a*R*)-9-(Hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,7,10,10a-tetrahydrobenzo[c]chromen-1-ol (HU-210) is a synthetic cannabinoid, with a classical cannabinoid structure similar to Δ^9-tetrahydrocannabinol (Δ^9-THC). In this study, the *in vitro* metabolism of HU-210 was investigated in human liver microsomes to characterize associated phase I metabolites. HU-210 was incubated with human liver microsomes, and the reaction mixture was analyzed using LC–MS/MS. HU-210 was metabolized in human liver microsomes, yielding about 24 metabolites. These metabolites were structurally characterized on the basis of accurate mass analyses and MS/MS fragmentation patterns. The major metabolic route for HU-210 was oxygenation. Metabolites M1–M7 were identified as mono-oxygenated metabolites; M8–M15, mono-hydroxylated metabolites; M16–M20, di-oxygenated metabolites; and M21–M24, di-hydroxylated metabolites. These results provide evidence for *in vivo* HU-210 metabolism, and they may be applied to the analysis of HU-210 and its relevant metabolites in biological samples.

1. Introduction

(6a*R*,10a*R*)-9-(Hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,7,10,10a-tetrahydrobenzo[c]chromen-1-ol (HU-210, Fig. 2), a synthetic cannabinoid, is structurally and pharmacologically similar to Δ^9-tetrahydrocannabinol (Δ^9-THC), the main active ingredient in marijuana. This compound was first synthesized from (1R,5S)-Myrtenol in 1988 [1]. Recently, it has been found in the herbal mixture "Spice", sold in the European countries [2,3]. HU-210 is a schedule I controlled substance in the United States [4].

Synthetic cannabinoids fall in a wide range of structural classes, and are mainly categorized into 2 groups: the classical structures related to THC, and the non-classical structures such as aminoalkylindole, 1,5-diarylpyrazole, quinolines, arylsulfonamides, and eicosanoids [5]. As shown in Fig. 2, HU-210 is categorized as a THC substance, and shows Δ^9-THC-like pharmacological activities [6–8]. *In vitro* studies show that HU-210 binds to both the brain cannabinoid receptor CB_1 and the peripheral cannabinoid receptor CB_2, with higher affinity than Δ^9-THC [9].

The metabolism of Δ^9-THC and the synthetic cannabinoid analogs such as JWH-015, JWH-018, and JWH-210 has been extensively studied by many groups [10–16]. However, to our knowledge, the chemical structures of HU-210 metabolites have not yet been elucidated. The aim of this study was to identify the phase I metabolites of HU-210 in human liver microsomes using high-resolution mass spectrometry (HR-MS) and collision-induced dissociation (CID) in MS/MS analysis.

2. Materials and methods

2.1. Chemicals and materials

HU-210 (98%) was purchased from Tocris Bioscience (Bristol, UK). HPLC-grade methanol and acetonitrile were purchased from J.T. Baker (Philipsburg, NJ, USA). β-Nicotinamide adenine dinucleotide phosphate sodium salt (β-$NADP^+$), glucose 6-phosphate, glucose-6-phosphate dehydrogenase, formic acid, KH_2PO_4, and K_2HPO_4 were obtained from Sigma–Aldrich Co. LLC (St. Louis, MO, USA). Human liver microsomes were purchased from BD Biosciences (Woburn, MA, USA). Solid-phase extraction (SPE)

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cartridge (OASIS HLB, 1 ml, 30 mg) was purchased from Waters (Milford, MA, USA). Deionized water was prepared from a Millipore Direct-Q UV system (Millipore, Milford, MA, USA). All chemicals and reagents used in this study were of analytical grade.

2.2. Biotransformation of HU-210 in human liver microsomes

HU-210 was incubated with human liver microsomes for 2 h. The 1 ml incubation mixture consisted of 1 mg protein/ml of human liver microsomes, 100 mM potassium phosphate buffer (pH 7.4), and 12.5 μg/ml HU-210. The NADPH-generating system (NGS) consisted of 1 unit of glucose-6-phosphate dehydrogenase, 0.1 M glucose-6-phosphate, and 10 mg/ml β-$NADP^+$. The reaction was started by adding NGS, and stopped by adding equal volumes of ice-cold 0.1% formic acid after a 2 h incubation period. The temperature was maintained at 37 °C during the entire incubation time.

2.3. Sample preparation

After incubation, the reaction mixtures were percolated through SPE cartridges. The SPE method involved the following steps. First, the SPE cartridge was activated with 1 ml of methanol, and equilibrated with 1 ml of deionized water. Then, the sample was loaded onto the SPE column, and the column was washed twice with 1 ml of deionized water. The reaction mixture was eluted with 1 ml of methanol, and evaporated under a stream of nitrogen at 55 °C. The residue was reconstituted with 100 μl of 70% methanol.

2.4. LC–MS/MS analysis

An LTQ-Orbitrap LC–MS/MS system (Thermo Fischer Scientific, Waltham, MA, USA) was used to identify HU-210 metabolites. The LC system was equipped with a solvent delivery pump, degasser, autosampler, and column thermostat. The separation was performed on a Hypersil Gold C_{18} (150 mm × 2.1 mm, 3 μm; Thermo

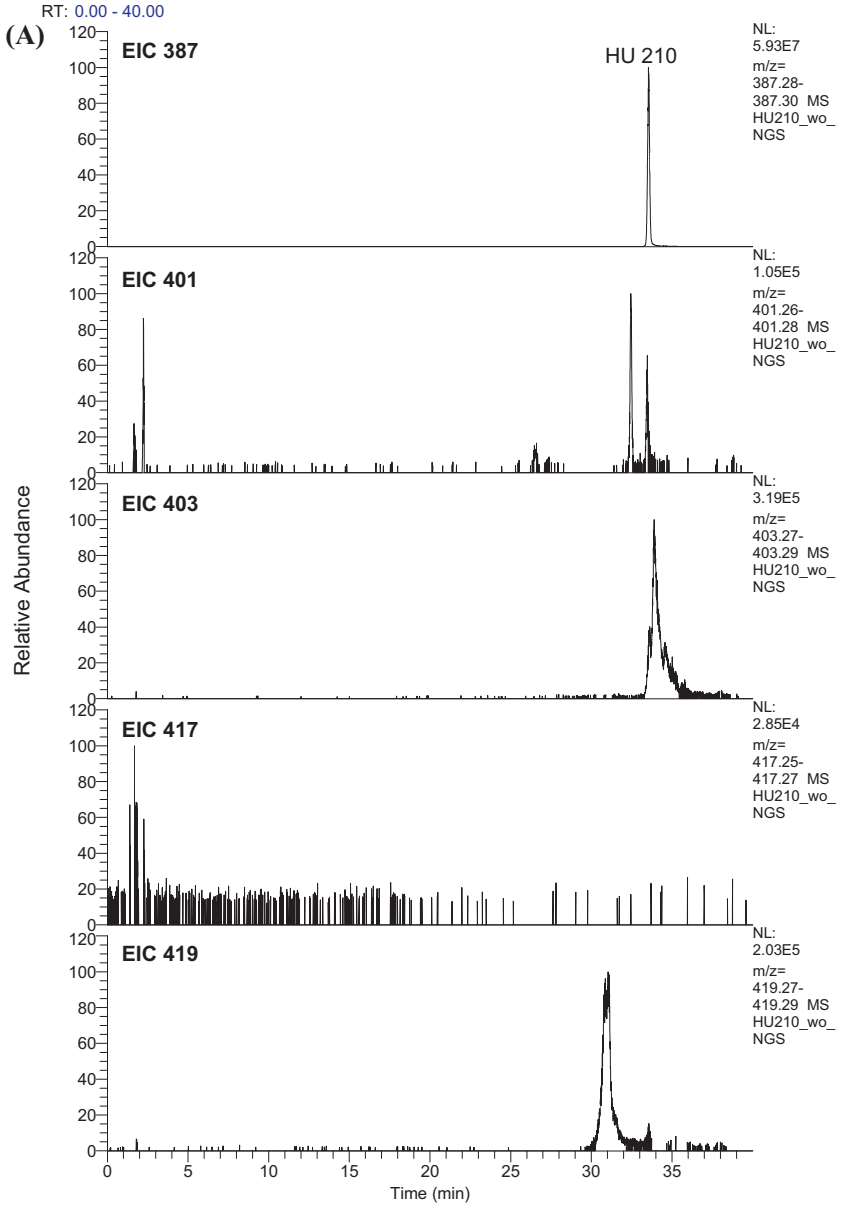

Fig. 1. Extracted ion chromatograms of HU-210 and its metabolites in human liver microsomes (A) without and (B) with NADPH.