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Synthesis and identifications of potential metabolites as biomarkers of the synthetic cannabinoid AKB-48

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ABSTRACT

AKB-48 belongs to the family of synthetic cannabinoids. It has strong binding affinity to CB_1 receptor and is psychoactive. It is banned in many countries including USA, Japan, Germany, New Zealand, Singapore and China etc. But the difficulty in detecting the parent compound in urine samples highlights the importance of studies of its metabolites. Here we report the synthesis of 19 potential metabolites of AKB-48, among which, compounds **2**, **9**, **10**, **30** and **31**, together with the commercially available substance **5** were identified as metabolites of AKB-48 by comparison with one authentic human urine sample and human liver microsomal data. Compounds **10** and **30** could be of use as biomarkers in detecting AKB-48 in human urine samples.

1. Introduction

Synthetic cannabinoids have emerged and grown to become a common substitute to cannabis in recent years. The rate at which old synthetic cannabinoids are replaced on the market with new ones as they become classified as harmful substances puts great pressure on forensic laboratories and governments. Synthetic cannabinoids are not always detected in routine cannabinoid urine screening methods; however, their metabolites can be detected and are therefore important to enable identifications of synthetic cannabinoid intakes in analysis of urine samples. As a member of the synthetic cannabinoid family, AKB-48 (Fig. 1) (APINACA) has stronger binding affinity to CB_1 receptor compared to tetrahydrocannabinol,[1] and is used as an alternative to cannabis. It is banned in many countries including USA, Japan, Germany, New Zealand, Singapore and China etc.

AKB-48 was first reported by a Japanese group in 2012 as an ingredient in a synthetic cannabis smoking blend.[2] In 2013 the study of its metabolites was carried out by Gandhi et al. using human hepatocytes and high-resolution mass spectrometry.[3] Further investigation by Holm et al. found that CYP3A4 mediated the oxidative metabolism of AKB-48.[4] The oxidative metabolism happened mainly on the pentyl chain and the adamantyl moiety giving mono-, di- or tri-hydroxylated metabolites together with metabolites as ketones or carboxylic acids. Vikingsson et al. also put great effort in identification of AKB-48 metabolites using human liver microsomes and time of flight mass spectrometry.[5] Based on these research groups' findings more knowledge of AKB-48's metabolism is well described. However, the exact structure of the metabolites cannot be determined by LC-MS/MS alone. In a previous study we have synthesized and identified an interesting and important metabolite of AKB-48, with a secondary alcohol on the adamantane ring.[6] This is uncommon because metabolism of the adamantyl moiety normally leads to metabolites with a tertiary alcohol at the bridge-carbon as major.[7] Apart from the adamantyl moiety that is found in several synthetic cannabinoids, such as AB-001, SDB-001, STS-135; AKB-48 also contains a pentyl chain, which is an even more common moiety in other synthetic cannabinoids. There are some commercially available synthetic cannabinoid metabolites with a hydroxyl group at position 4 or 5 of the pentyl chain (Fig. 1), but unambiguous structure identification is lacking for several major metabolites.[5] To the best of our knowledge, there

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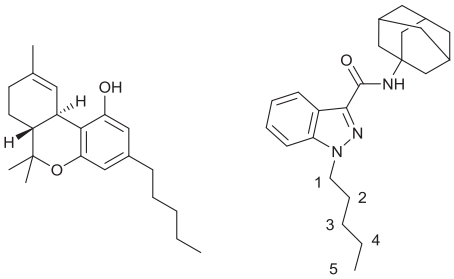


Fig. 1. Structures and activities of AKB-48 and tetrahydrocannabinol.

are not any synthesis and identification studies on the metabolic position of the pentyl chain of synthetic cannabinoids. To have a more complete picture of the metabolite profile of AKB-48, we continued our study of the synthesis and identifications of other metabolites of AKB-48. By comparison with an authentic urine sample we can identify its major metabolites, which could eventually lead us to discover better biomarkers for AKB-48, or even for other synthetic cannabinoids containing a pentyl chain or an adamantane moiety, in urine samples.

2. Results and discussion

According to our previous human liver microsome results[5] and the research by Gandhi et al.,[3] the oxidative metabolism of AKB-48 gave metabolites in the form of mono- or di-hydroxylations of the pentyl chain and/or of the adamantyl moiety. Therefore, the synthesis of potential metabolites of AKB-48 was focused on adding hydroxyl group(s) on its pentyl and/or adamantyl side chains. Furthermore, the synthetic compounds were used as references in the analysis of an authentic urine sample.

Compound **2** was synthesized using 3-indazole carboxylic acid **1** and 1-adamantanamine hydrochloride as starting materials with EDC as the coupling reagent in 48% yield. The yield was unfortunately low because of incomplete conversion.

Compounds **4** and **5** are commercially available. They can also be synthesized using hydroboration-oxidation, which was described below for the synthesis of compounds **19** and **20**. Alkylation of **2** with 5-bromopent-1-ene followed by dihydroxylation with OsO_4/NMO to give compound **6** (Scheme 1). Similar methods were applied to the synthesis of mono- or di-hydroxylations of the pentyl side chain at different position from compound **7** to give compounds **8**, **9** and **10**, and from **17** to give **18**, **19** and **20**, and from compound **21** to **22**, **23**, and **24**, which are shown in Scheme 2 and 4. The yield of compound **10** was low, and the purification of its mixture with compound **9** was difficult. It's probably easier to achieve **10** by reduction of compound **11** with $NaBH_4$ instead.

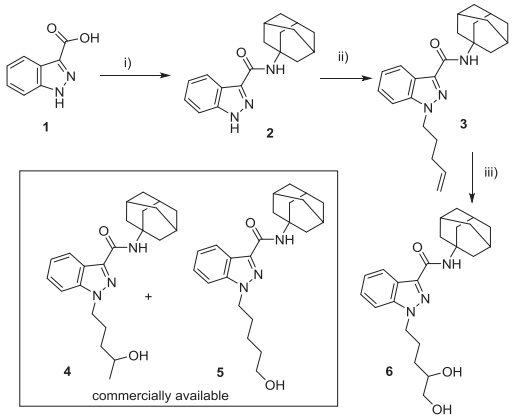
The ketones **11** and **12** were synthesized by microwave irradiation of the mixture of compound **2** or **15** and 1-chloro-3-pentanone in THF with t-BuOK as base at 120 °C for one hour (Scheme 3). Full conversion was not achieved, resulting in low yields, 24% and 28% respectively. Unfortunately, we were not able to find compound **11** or **12** in the authentic urine sample. Therefore, the optimization was not continued.

The adamantyl moiety of AKB-48 was prone to be oxidized by CYP3A4.[2,4] 1-adamantylamine **13** was oxidized instead with concentrated H_2SO_4/HNO_3 to give compound **14** containing a hydroxyl group.[8] With **14** as starting material other potential metabolites of AKB-48, compounds **12**, **15**, **16**, **18**–**20** and **22**–**24** were synthesized using similar methods as described above, showed in Scheme 4. When compound **15**, a similar structure to **2** was synthesized, another amide coupling reagent TBTU was used instead, and a good yield (81%) was achieved compared to that of EDC (27%) or DCC (31%). Hydroboration-oxidation of **17** gave a mixture of alcohols **19** and **20**, where the yield of **20** was very low (3%) because of anti-Markovnikov's rule and therefore there were also difficulties in the purification. Another synthetic route was developed to achieve **20** with better yield (49%, two steps) by first epoxidation of **17** with m-CPBA, followed by reduction of the resulting epoxide with $NaBH_4$ in *i*-PrOH at 60 °C for 3 h.

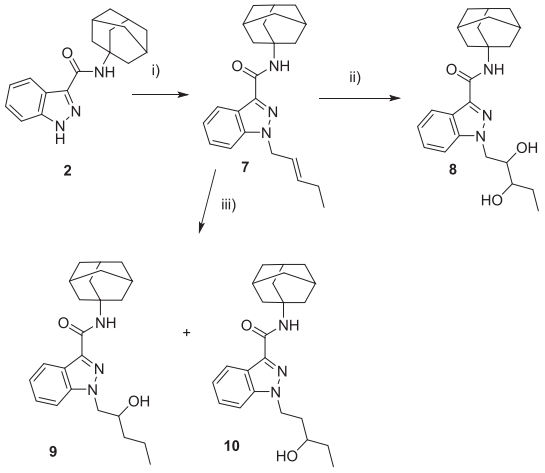
Although the tertiary carbons of the adamantyl moiety were much easier to be oxidized compared to the secondary ones with concentrated H_2SO_4/HNO_3, it's difficult to say whether it will be the same in vivo. Compound **25** was used as starting material to synthesize other potential metabolites with a secondary hydroxyl group. Conditions for a modified Ritter reaction reported in the literature were used.[9] The synthesis of compounds **25**–**31** was reported in our previous study.[5] However, it was not discussed in detail. But the synthetic route is shown in Scheme 5 for a better overview.

Compound **32** was also synthesized from compound **19** with Jones reagent in 59% yield (Scheme 6).

As described previously,[6] compound **30** was shown by LC-MS/



Scheme 1. i) EDC, HOBt, Et_3N, DMF, rt, overnight, 48%; ii) 5-bromopent-1-ene, *t*-BuOK, DMF/THF (1:5), rt, overnight, 87%; iii) OsO_4 (aq), NMO, THF/H_2O (3:1), rt, overnight, 66%. Compounds **4** and **5**, commercially available.



Scheme 2. i) 1-bromopent-2-ene, t-BuOK in DMF/THF (1:5), rt, overnight, 98%; ii) OsO4 (aq), NMO, THF/H2O (3:1), rt, overnight, 80%; iii) a) BH3·THF, N2 (g), 0 °C, 2 h. b) NaOH (aq), H2O2, rt, overnight, 9, 69%; 10, 8%.