Synthesis of JWH-250 4-Hydroxypentyl Metabolite

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Introduction

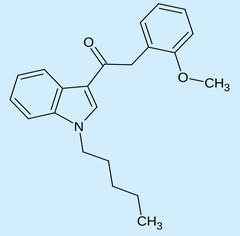
JWH-250 (1-pentyl-3-(2-methoxyphenylacetyl)indole) is one compound in a family of cannabimimetic indoles that shows a high-affinity for both the central cannabinoid (CB1) and the peripheral cannabinoid (CB2) receptors.[1] JWH-250 was discovered by Dr. John W. Huffman who created JWH-250 and a number of other compounds to research the structure and function of the endocannabinoid system of mammals.[2]

In recent times, these indole cannabimimetics, commonly known as “spice,” have been sold as “legal highs” as a substitute for marijuana and are now coming under regulatory control. The indole cannabimimetics are metabolized to a variety of hydroxylated and carboxylated derivatives.[3]

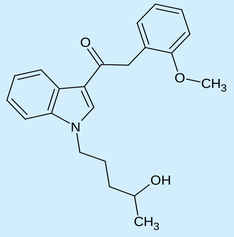
Identification and quantitation of these drugs in forensic and clinical samples has clinical and societal ramifications and can impact forensic investigations and patient treatment decisions. Certified reference standards are required in order to accurately identify and quantitate these cannabimimetic drugs and their metabolites.

Synthesis

Synthesis of the 4-hydroxypentyl metabolite of JWH-250 (1-(4-hydroxypentyl)-3-(2-methoxyphenylacetyl)indole) presented unique challenges compared to the 3-naphthoyl series of JWH indoles. In JWH-250, the 3-naphthoyl substituent on the indole is replaced with a 2'-methoxyphenylacetyl group, with markedly different reactivity of the indole nitrogen and a potential for side reactions at the acetyl-alpha carbon position. JWH-250 4-hydroxypentyl metabolite is a major metabolite of JWH-250. The aim of this work was to synthesize & optimize the processes for JWH-250 4-hydroxypentyl metabolite through a four step synthetic scheme. Analysis and characterization of JWH-250 4-hydroxypentyl metabolite were accomplished via HPLC, Mass Spectra, and ^{1}H-NMR.



JWH-250 1-pentyl-3-(2-methoxyphenylacetyl)indole



JWH-250 4-Hydroxypentyl Metabolite

References

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2. Bioorganic & Medicinal Chemistry Letters, 2005, 15 , 4110–13.
3. Front Behav Neurosci, 2011, 5, 60.
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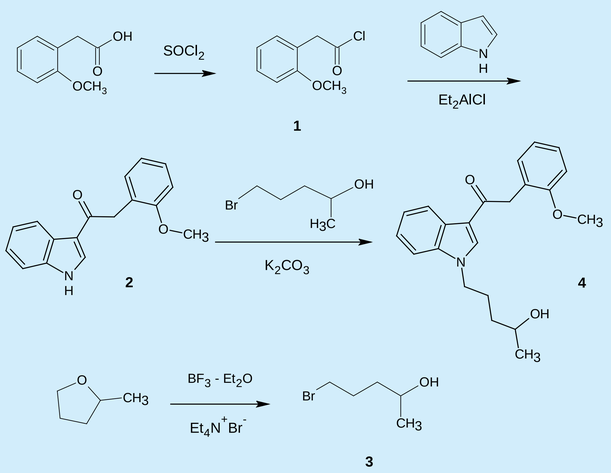
Synthetic Scheme for JWH-250 4-Hydroxypentyl Metabolite



Results & Discussion

There are two main literature approaches to synthesis of this class of compounds: 3-acylation of the indole followed by N-alkylation, and N-alkylation followed by 3-acylation.[2,4] We selected the former because we have previously successfully synthesized JWH-018 4-hydroxylpentyl metabolite at Cerilliant following this route. Several papers report using naphthoyl or aroyl chlorides for acylation of indoles to afford 3-acylindoles. The yields of this step are quite variable, and the products are difficult to purify.[1]

Synthesis of JWH-250 4-hydroxypentyl metabolite **4** started with 2-methoxyphenylacetic acid, which was reacted with thionyl chloride to form 2-methoxyphenylacetic chloride **1** in quantitative yield. This was followed by acylation of indole at the 3-position in the presence of diethylaluminum chloride in CH_2Cl_2 to obtain intermediate **2.** Acylation at the 3-position proceeded regioselectivity without NH protection. Compound **2** was purified by normal phase silica gel MPLC chromatography. Significant mass loss was observed during purification. Overall conversion was >90%, but an isolated yield of 15% to 25% was observed.

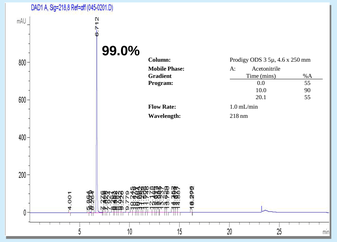
1-Bromo-4-pentanol **3** was prepared directly from 2-methyltetrahydrofuran by regioselective cleavage in which advantage was taken of the different steric environments of the two oxygen-carbon bonds.[5]

Problems were associated with the key step: indole-N-substitution of 1-Bromo-4-pentanol **3**. Initially we ran the reaction of compounds **2** & **3** in the presence of excess K_2CO_3 at 50°C.[5] The expected alkylation was very slow, and we observed only a small percentage of compound **4** with a large proportion of unreacted indole **2**. Unlike other N-alkyl substitution reactions reported with related 3-benzoyl or napthoyl substituted indoles,[6] the NH in compound **2** has poor reactivity due to its less rigid structure and weaker acidity. The reaction required control of reaction temperature between 0°C and room temperature and addition of multiple small portions of 1-Bromo-4-pentanol **3** and K_2CO_3 into the reaction mixture. Conversion to the product **4** was improved from <10% to >40% .

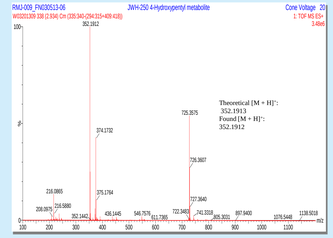
Synthesis of JWH-250 4-hydroxypentyl metabolite was completed with a 10% overall yield. Characterization was accomplished via HPLC, Mass Spectra, and ^{1}H NMR. Stability of the product at low concentration was then investigated for development of a certified solution reference standard.

Analytical Data and Preparation of Solution Standard for JWH-250 4-Hydroxypentyl Metabolite

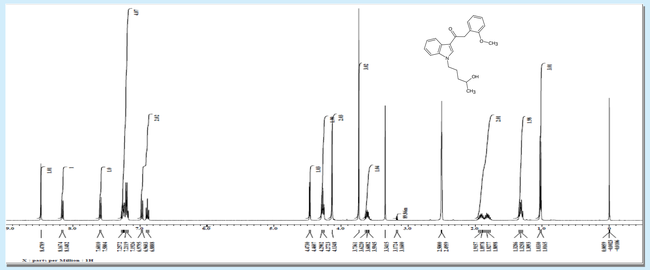
HPLC



HRMS



^{1}H NMR



A solution standard of JWH-250 4-hydroxypentyl metabolite was prepared at 1.0 mg/mL in acetonitrile (ACN) and methanol. Stability was evaluated by HPLC over a one week time frame under four different storage temperatures. The HPLC results showed that JWH-250 4-hydroxypentyl metabolite is stable as a standard solution in both acetonitrile and methanol, giving us flexibility in choosing the solvent for the solution standard. Results for ACN are shown in Table 1.

Table 1. Stability of JWH-250 4-hydroxypentyl metabolite in ACN at 1.0 mg/mL

Analysis Time	Freezer	Refrigerator	Room Temp.	40°C
T = 0 day	99.70%	99.70%	99.69%	99.69%
T = 3 days	99.70%	99.59%	99.70%	99.70%
T = 7 days	99.69%	99.40%	99.69%	99.70%

Conclusions

- Synthesis of JWH-250 4-hydroxypentyl metabolite was completed in four synthetic steps.
- The yield of the key final step was improved through control of temperature and reagent addition.
- The product was certified and developed into a stable solution-based Certified Reference Material for identification and quantitative applications.