Cannabinoid Receptor Bioassay:

A Characterization of UR-144, XLR-11, Their Metabolites and Degradants

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Introduction

Synthetic cannabinoids are an exponentially diverse group of designer drugs that have received global attention in recent years. This particular class of drugs has become popular amongst users due to the cannabimimetic high they offer,[1] even though no studies exist that demonstrate the safety of these drugs when consumed by humans.[5] Their great variety in structure makes it difficult for the law to determine if they should be controlled or not, especially with the current lack of information regarding the cannabimimetic nature of many synthetic cannabinoids. The cannabimimetic effects synthetic cannabinoids generally possess are attributed to the G protein-coupled receptors (GPCRs), referred to as CB1 and CB2, that synthetic cannabinoids and marijuana (Δ^9-tetrahydrocannabinol) both interact with in the body.[2] CB1 receptors, found in the central nervous system, are associated with hallucinogenic effects, while the CB2 receptors, found in the peripheral nervous system, are linked to therapeutic effects.[3]

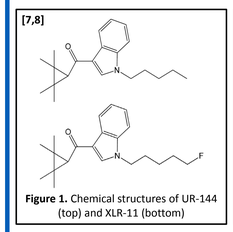

Figure 1. Chemical structures of UR-144 (top) and XLR-11 (bottom)

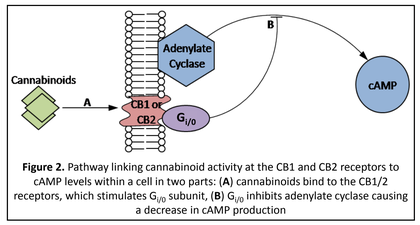

Figure 2. Pathway linking cannabinoid activity at the CB1 and CB2 receptors to cAMP levels within a cell in two parts: (**A**) cannabinoids bind to the CB1/2 receptors, which stimulates $G_{i/o}$ subunit, (**B**) $G_{i/o}$ inhibits adenylate cyclase causing a decrease in cAMP production

Project Goal

- Research Goal: evaluate the potencies of different synthetic cannabinoids, including the UR-144 and XLR-11 family (Figure 1) at the CB1 and CB2 receptors
  - Monitor cyclic adenosine monophosphate (cAMP) levels in cells due to the pathway in Figure 2[6]
    - Proprietary mammalian cell-based cannabinoid receptor bioassay developed at Aegis Sciences Corporation®
  - Generate dose-response curves from data and use Equation 1 to calculate the potency (EC_{50}) of the synthetic cannabinoid compounds

$$Y = \frac{a-d}{1+(X/c)^b} + d \qquad (1)$$

    - Equation 1: Y = response, X = concentration of drug used, a = lower asymptote of the dose-response curve, b = curve's slope factor, d = curve's upper asymptote, and c = curve's EC_{50} value[9]
  - EC_{50} value indicate the amount of the drug needed to reach the half-maximal effect, therefore, compounds with smaller EC_{50} values are considered as more potent than their counterparts

Methods

- Cannabinoid Receptor Bioassay:
  - Mammalian cell-based bioassay property of Aegis Sciences Corporation®
- Drug Standards: One Cerilliant© and eleven Cayman Chemical© drug standards (Table 1) in the UR-144 and XLR-11 family of synthetic cannabinoids
- Characterization:
  1. Chinese hamster ovary (CHO) cells expressing either CB1 or CB2 receptors plated into 96-well half-area plates
  2. Stimulated cells with forskolin (FSK) and sixteen varying concentrations of drug standards, ranging from as high as 150 µg/mL to 0 µg/mL, in triplicate
  3. cAMP standards plated on separate 96-well half-area plate
  4. cAMP levels measured for all plates with optimized version of the Perkin Elmer© LANCE® *Ultra* cAMP Assay kit[10] (Figure 3)
  5. Data analysis performed with GraphPad *Prism 6* software[11]
     - Applied Equation 1 to dose-response curves to find EC_{50}

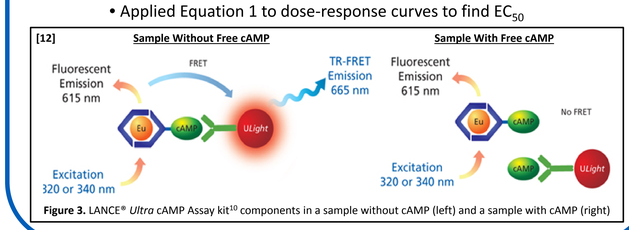

Figure 3. LANCE® *Ultra* cAMP Assay kit[10] components in a sample without cAMP (left) and a sample with cAMP (right)

Abstract

Synthetic cannabinoids, one of the largest growing and widely varying groups of designer drugs, have become popular in recent years due to the cannabimimetic high they offer to users.[1] The similarities in the effects of synthetic cannabinoids and marijuana (Δ^9-tetrahydrocannabinol) are thought to be the result of these compounds interacting with the same G protein-coupled receptors (GPCRs).[2] These GPCRs are more commonly referred to as the cannabinoid binding receptors, CB1 and CB2, and are located in the body's central and peripheral nervous systems, respectively. Due to their separate locations, CB1 receptors are generally associated with the hallucinogenic effects of cannabinoids, while the CB2 receptors are linked to the therapeutic effects of cannabinoids.[3] However, not much has been discovered regarding the potencies of these compounds at these receptors. This lack of information regarding the cannabimimetic nature of these drugs makes it difficult for authorities to schedule them. In order to learn more about how different synthetic cannabinoids interact with the CB1 and CB2 receptors, the potency (EC_{50}) of two of these synthetic cannabinoids, UR-144 and XLR-11, as well as ten of their metabolites and degradants, was investigated using a mammalian cell-based cannabinoid receptor bioassay. For UR-144, EC_{50} values of 8.5 ng/mL and 3.6 ng/mL were found for the CB1 and CB2 receptors, respectively. Two of the remaining UR-144 compounds, the UR-144 degradant and the N-(2-hydroxypentyl) metabolite, were determined to be more potent at the CB1 receptors, while the N-(4-hydroxypentyl) and N-(5-hydroxypentyl) metabolites both were found to be more potent than UR-144 at the CB2 receptors. With XLR-11, the CB 1 and CB2 EC_{50} values were found to be 101 ng/mL and 6.6 ng/mL, respectively. All three XLR-11 metabolites and degradants tested proved to be more potent than XLR-11 at the CB2 receptors, with one of these three compounds being more potent at the CB1 receptors as well. Combining the knowledge that seven of the ten metabolized and degraded forms of UR-144 and XLR-11 tested demonstrated greater potencies than the parent compounds, and the fact that the metabolized and degraded forms are likely to be more commonly seen in forensic toxicological samples than UR-144 and XLR-11 themselves, it can be suggested that the bioassay shows great potential as a screening method for toxicological samples. In conclusion, this study's results support the claim that several of the UR-144 and XLR-11 compounds are cannabimimetic due to their activity with the CB1 and CB2 receptors. This data is not only applicable to forensics by helping determine if these drugs should continue to be scheduled, but it can also be useful to the field of medicinal chemistry where cannabinoids with a greater potency at the CB2 receptors than the CB1 receptors are being investigated as potential therapeutic treatments.[4]

Results

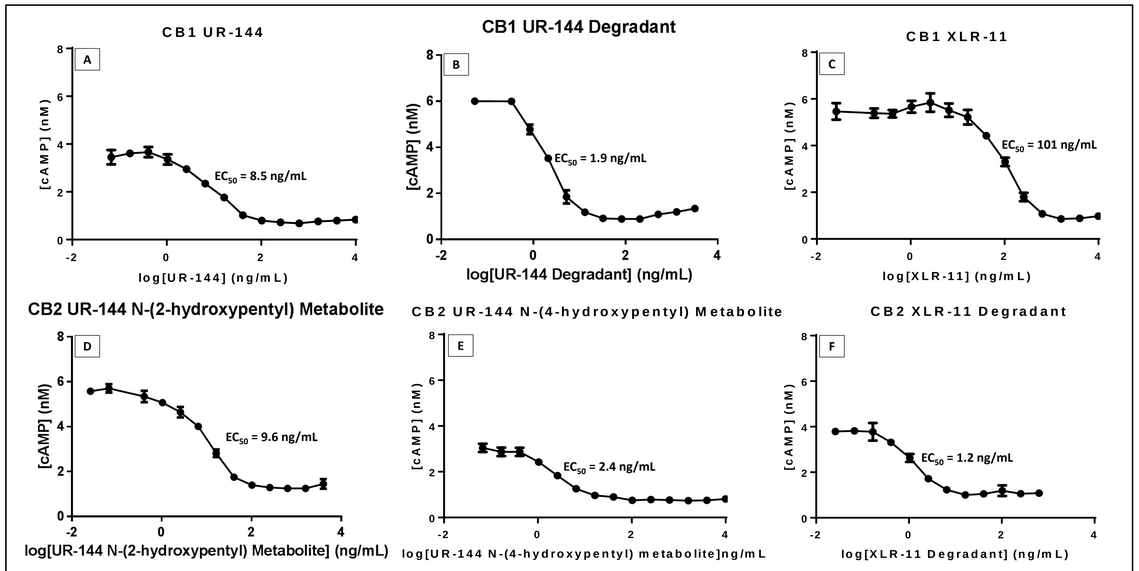

Figure 4. Representative characterization dose-response curves for the CB1 receptors with UR-144 (A), UR-144 degradant (B) and XLR-11 (C), and for the CB2 receptors with UR-144 N-(2-hydroxypentyl) metabolite (D), UR-144 N-(4-hydroxypentyl) metabolite (E), and XLR-11 degradant (F)

Table 1. Potency rankings of the dose-response curve results for UR-144, XLR-11, their metabolites and degradants, including calculated EC_{50} concentrations and R^2 values
*EC_{50} values greater than 1000 ng/mL are reported as 'No Activity'

CB1			Potency Ranking	CB2		
R^2	EC_{50} (ng/mL)	Compounds		Compounds	EC_{50} (ng/mL)	R^2
0.99	1.9	UR-144 degradant	**1**	UR-144 N-(5-hydroxypentyl) metabolite	0.62	0.99
0.96	2.1	XLR-11 degradant	**2**	XLR-11 degradant	1.2	0.98
0.99	2.5	UR-144 N-(2-hydroxypentyl) metabolite	**3**	XLR-11 4-hydroxypentyl metabolite	1.9	0.99
0.99	8.5	UR-144	**4**	UR-144 N-(4-hydroxypentyl) metabolite	2.4	0.99
0.99	101	XLR-11	**5**	UR-144	3.6	0.99
0.99	183	XLR-11 6-hydroxyindole metabolite	**6**	XLR-11 6-hydroxyindole metabolite	4.7	0.99
0.97	231	UR-144 N-(4-hydroxypentyl) metabolite	**7**	UR-144 degradant	6.3	0.99
0.98	250	XLR-11 4-hydroxypentyl metabolite	**8**	XLR-11	6.6	0.97
0.99	273	UR-144 N-(5-hydroxypentyl) metabolite	**9**	UR-144 N-(2-hydroxypentyl) metabolite	9.6	0.99
0.96	No Activity	Tie: UR-144 degradant N-pentanoic acid metabolite; UR-144 N-(5-hydroxypentyl) β-D-glucoronide; UR-144 N-pentanoic acid metabolite	**10**	UR-144 N-(5-hydroxypentyl) β-D-glucoronide	59	0.96
0.98	No Activity		**11**	UR-144 N-pentanoic acid metabolite	219	0.99
0.98	No Activity		**12**	UR-144 degradant N-pentanoic acid metabolite	No Activity	0.99

Discussion and Conclusions

From analyzing the results of the characterization study (Table 1), three key findings became apparent:

- Key Finding #1: majority of metabolites and degradants explored in study demonstrate activity
  - XLR-11: all three metabolites and degradants demonstrated activity at both CB1 and CB2 receptors
  - UR-144:
    - Four metabolites and degradants demonstrated activity at CB1 receptors
    - Six metabolites and degradants demonstrated activity at CB2 receptors
    - Hold-out: UR-144 degradant N-pentanoic acid metabolite was deemed to show no activity at neither CB1 nor CB2 receptors
  - **Implication**: bioassay may be powerful screening tool for presence of synthetic cannabinoids in forensic samples because metabolized and degraded forms are more common in these types of samples
- Key Finding #2: most compounds tested had lower EC_{50} values at CB2 receptors
  - XLR-11: includes all four XLR-11 compounds
  - UR-144: includes five of seven UR-144 compounds
    - Hold-outs: both the UR-144 degradant and UR-144 N-(2-hydroxypentyl) metabolite had lower EC_{50} values at the CB1 receptors
  - **Implication**: UR-144 and XLR-11 compounds can be considered generally more therapeutic than hallucinogenic, which has made them potential targets for alternative therapies
- Key Finding #3: some metabolites and degradants demonstrated greater potencies than their parent compound
  - XLR-11:
    - XLR-11 degradant at the CB1 receptors
    - All three metabolites and degradants at the CB2 receptors
  - UR-144:
    - UR-144 degradant and UR-144 N-(2-hydroxypentyl) metabolite at the CB1 receptors
    - UR-144 N-(4-hydroxypentyl) metabolite and UR-144 N-(5-hydroxypentyl) metabolite at the CB2 receptors
  - **Implication**: bioassay could be useful for evaluating relative potencies of lesser-understood cannabinoids and compare them to more well-understood cannabinoids like THC

In the future, the bioassay characterization work may be continued to include some of the more recent sub-classes of synthetic cannabinoids, such as the AB-PINACA series and other fourth generation synthetic cannabinoids.

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