**PROJECT TITLE:** Determination of CB1 Binding and Agonist Activity of Emerging Synthetic Cannabinoid Compounds

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The United State Congress passed The Synthetic Drug Abuse Prevention Act on July 9, 2012, which is the most recent attempt by the United States government to control synthetic drugs, including cannabinoids. This legislation places synthetic cannabinoids into Schedule I of the Controlled Substances Act (21 U.S.C. 812(c)) based on structure, receptor binding, and function. There are currently at least 17 chemical classes of cannabinoid structure known with all but two reported in the literature in seized samples prior to 2015. A number of these reported cannabinoids have cores or substitutions that fall outside of the definitions of an analog under current regulations and it is unknown if they are cannabinoid receptor 1 (CB1) agonists. It is critical that CB1 receptor binding affinities and activities be assessed for new synthetic cannabinoid compounds. Because standards for many of these new compounds are often unavailable from reliable sources, a number of the cannabinoid core structures will be synthesized. All proposed syntheses will use well established literature methods involving few steps and commercially available materials. GC/MS, NMR, Raman, and IR spectroscopies will be used to confirm the correct compounds have been synthesized. These compounds will then be used to perform receptor binding assays and functional studies to determine their potential as CB1 receptor agonists. We propose a liquid chromatography-mass spectrometry-mass spectrometry (LC/MS/MS) method using deuterated rather than radioactive compounds to determine receptor binding affinities to possibly decrease cost, time and safety concerns currently associated with radioligand binding studies. These binding assays will initially be performed in tandem with the standard radioligand methods to determine the efficiency of the method. CB1 receptor activity will be determined by measuring the ability of our test compounds to inhibit forskolin-induced cAMP levels. Cannabinoid receptors are G-protein coupled receptors that link predominately to Gi/Go, thus their activation is linked to a reduction in cAMP levels within the cell. By measuring the effects of the compounds on forskolin induced cAMP, we should be able to determine the action of the compounds on CB1 receptors. The resulting analytical data and methodologies outlined in this proposal represent important advancements in forensic chemistry and toxicology by fulfilling the gap in research necessary to define a compound as a CB1 receptor agonist and thus part of the requirements for placing it into Schedule 1 of the Controlled Substances Act.