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A structure–reactivity relationship driven approach to the identification of a color test protocol for the presumptive indication of synthetic cannabimimetic drugs of abuse

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ABSTRACT

The number of analyses of synthetic cannabimimetic drugs of abuse by forensic laboratories in the United States grew rapidly from 2010 to 2012 and then declined somewhat in 2013. In 2010, according to the National Forensic Laboratory Information System (NFLIS), 3,287 reports by federal, state and local forensic laboratories were identified as containing synthetic cannabinoids. In 2011 and 2012, the numbers increased to 23,693 and 42,503, respectively. 27,119 reports were identified in 2013. Several commonly encountered structural sub-classes of these synthetic designer drugs, namely the naphthoylindoles, benzoylindoles, phenylacetylindoles, and cyclopropoylindoles contain a ketone functional group. The Duquenois-Levine color test for the presumptive identification of classical cannabinoids such as Δ^9-tetrahydrocannabinol is negative for the synthetic cannabimimetics. The van Urk color test for the presumptive identification of indole containing drugs of abuse is also negative for these compounds. The use of 2,4-dinitrophenylhydrazine as an alternative color test reagent (targeting the keto moiety rather than the indole) for presumptive identification of these classes of drugs was investigated.

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1. Introduction

Marijuana (*Cannabis sativa* L.) is the world's most popular illicit drug of abuse [1]. Δ^9-Tetrahydrocannabinol (often referred to simply as THC) is the principal pharmacologically active constituent of marijuana and is responsible for this vaunted status [2]. Its well-known psychoactive properties are the physiological result of binding to, and subsequent agonism of the cannabinoid subtype 1 (CB1) receptor in the central nervous system (CNS). Δ^9-Tetrahydrocannabinol and structurally similar naturally occurring compounds (e.g. cannabidiol, cannabigerol, and cannabichromene) are typically referred to as classical cannabinoids (Fig. 1) [3].

The first appearance of non-marijuana, plant-based material surreptitiously marketed in smoking blends as "legal highs" and purported to contain "synthetic marijuana" dates back to at least 2006 [4,5]. Unlike traditional marijuana, "synthetic marijuana" could not be presumptively identified using the Duquenois–Levine color test [6]. As the popularity and proliferation of these products intensified throughout the illicit drug subculture, detailed analytical, chemical, and structural analyses demonstrated that these new products did not contain Δ^9-tetrahydrocannabinol or any classical cannabinoids, but rather, fully synthetic drugs which mimicked their binding to the CB1 receptor and evoked substantially similar psychoactive responses (i.e. cannabimimetics).

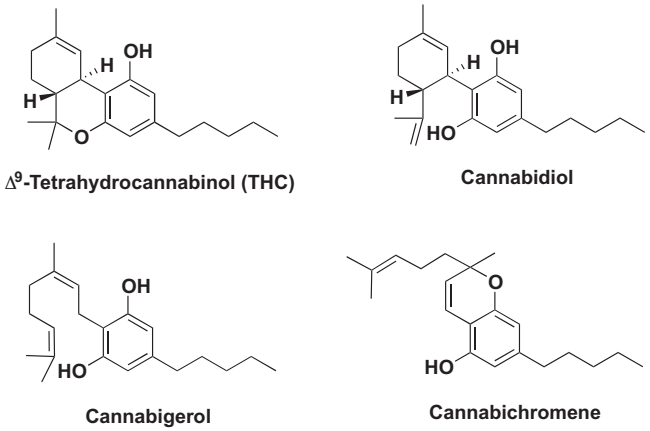

Fig. 1. Classical cannabinoids.

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These substances were not endogenous constituents of the marketed plant material, but rather, exogenous adulterants which had been adsorbed onto otherwise benign plant material.

In December of 2008, media reports indicated that scientists at the German pharmaceutical company, THC Pharma were the first to definitively identify JWH-018 (Fig. 2) as an active ingredient in commercial "legal high" plant-based products being sold for smoking purposes. JWH-018 is a member of a class of synthetic indole-derived CB1 receptor agonists designed and developed in the laboratories of Prof. John Huffman of Clemson University as part of a legitimate research effort to understand and exploit for beneficial medical purposes the dynamics of cannabinoid receptor binding interactions with novel synthetic ligands [7].

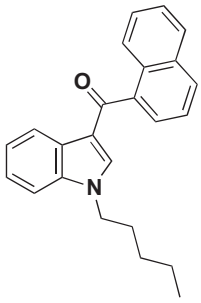

Fig. 2. Synthetic cannabinoid JWH-018.

The term "synthetic marijuana" was used effectively to market smoking plant blends containing synthetic cannabinoids in the first few years after their emergence. To a naive target audience this deliberate misnomer indicated marijuana, and by direct inference, Δ^9-tetrahydrocannabinol that had been synthetically produced. The more specific term synthetic THC was avoided by the purveyors of these products even though synthetic THC had existed in the form of the drug Dronabinol (which was approved in the United States by the Food and Drug Administration, FDA in 1986 as a treatment for chemotherapy induced nausea and vomiting). More recently, due to a combination of widespread passage of legislative bans incorporating the specific term "synthetic marijuana" and aggressive law enforcement efforts worldwide, the term has disappeared from vendor websites and current product labeling. Nevertheless, a keyword search of any general purpose or scientific publication database demonstrates that the term is already firmly rooted in current lexicon, which is proof, in and of itself, of the effectiveness of the original marketing efforts.

The commercial brand names of two popular early prototypes, "Spice" and "K2" have garnered such cult-like status among illicit drug users that these names have persisted in street vernacular and are often erroneously used to designate the entire class of drugs rather than specific commercial products containing individual members of the class. Nevertheless, from a strictly scientific perspective, all these substances are more accurately termed synthetic cannabimimetics or non-classical cannabinoids, terminology which will be used interchangeably throughout this manuscript. This manuscript will attempt to offer plausible steric- and electronic-based mechanistic rationalizations for observed structure–reactivity responses of Δ^9-tetrahydrocannabinol and synthetic cannabimimetics in the presence of selected color test reagents. The analyses will be used to identify a new color test protocol suitable for the presumptive indication of several structural classes of synthetic cannabimimetic drugs of abuse [8]. Both Fast Blue 2B and Liebermann's reagent have previously been reported as color test reagents for synthetic cannabinoids [9,10].

2. Structural and mechanistic rationale for color tests

In spite of the fact that the modern forensic laboratory employs gas chromatography–mass spectrometry (GC–MS) as the primary analytical technique for the confirmative identification of unknown substances, there is still a role for presumptive identification tests such as color tests. Color tests provide presumptive indication of the presence or absence of specific drugs, or more accurately, specific structural classes of drugs or specific functional groups embedded within particular drugs. When tasked with the analysis of exhibits containing multiple individual units, color tests may facilitate rapid and economical screening of said units so that more costly and time intensive analytical tests (such as GC–MS) may be more judiciously prioritized. With respect to synthetic cannabinoids, it is not unusual for law enforcement to seize hundreds or even thousands of individual packets of suspected illicit material in a single act of enforcement and submit them for analysis. In resource-limited environments, color tests may be integral to the routine processing of such suspected illicit drug evidence. One way to identify suitable color tests for new classes of compounds is to consider how and why established color tests work, or do not work from a structural/mechanistic point of view.

Most drugs of forensic interest possess "electron-rich" chemical substructures such as basic amines (e.g. cocaine, PCP), and oxygen- or alkyl-substituted benzene rings (e.g. MDMA, heroin). Most color test reagents used to presumptively indicate the presence of drugs of forensic interest contain "electron poor" chemical substructures such as metal cations (e.g. cobalt thiocyanate in the Scott test for cocaine) and aldehydes (e.g. formaldehyde in the Marquis test for heroin). Mixing the two can permit the "electron rich" drug component to donate electron density to the "electron poor" test component. If the energy associated with the electron transfer process corresponds to the visible region of the electromagnetic spectrum, the transfer may be visually manifested as a color change (positive test result). The electron transfer process may result in either sharing of electron density without physical bond formation (e.g. cobalt thiocyanate test for cocaine) or irreversible electron transfer with physical bond formation (e.g. the Marquis test for heroin) [11]. These basic principles can be illustrated in greater detail by considering the Duquenois–Levine color test for classical cannabinoids such as, Δ^9-tetrahydrocannabinol (Fig. 3) [6].

3. Structural requirements for Duquenois–Levine color test substrates

Δ^9-Tetrahydrocannabinol has at its core a benzene ring bearing four electron-donating substituents (two alkyl groups, one hydroxy group, and one alkoxy group) rendering it an electron rich molecule. Substituent alkyl groups inductively donate electron density to an appendant benzene ring [12]. Substituent hydroxy and alkoxy groups inductively withdraw electron density from, but simultaneously donate electron density to an appendant benzene ring via lone pair resonance, with the latter effect predominating [12]. The Duquenois–Levine reagent is a mixture of vanillin and acetaldehyde. After mixing with the test substrate, the characteristic positive purple response occurs upon subsequent addition of concentrated hydrochloric acid. Both vanillin and acetaldehyde contain carbonyl groups. A carbonyl group is polarized which renders the carbonyl carbon electron deficient. In the presence of a protic acid such as hydrochloric acid, the electron-poor nature of the carbonyl carbon is enhanced due to Lewis acid–Lewis base interactions. It has been theorized that